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ONSITE WASTEWATER TREATMENT SYSTEMS IN A CHANGING CLIMATE:
TECHNOLOGY ROBUSTNESS AND MECHANISMS

BY

JENNIFER ANNE COOPER

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

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UNIVERSITY OF RHODE ISLAND

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DOCTOR OF PHILOSOPHY DISSERTATION

OF

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ABSTRACT

Nearly 25% of U.S. households rely on onsite wastewater treatment system (OWTS), or septic systems, to renovate wastewater before it is recharged to groundwater. These systems rely on soil processes as the final step in contaminant removal. Reliance on soil microbial, physical and chemical processes, which are sensitive to environmental perturbations (e.g. changes in pH, temperature, moisture, O₂, presence of toxins), may result in variable wastewater treatment and release of contaminants to groundwater.

The extent of treatment in the soil treatment area (STA; also known as drainfield or leachfield) depends on the volume of unsaturated soil the wastewater must pass through, represented by the vertical separation between the infiltrative surface of the STA and the water table. Reduced treatment may result in greater transport of pathogens, nutrients (N and P), and biochemical oxygen demand (BOD₅) to groundwater, jeopardizing public and aquatic ecosystem health. The combined effects of climate change – warmer temperatures and elevated water tables due to sea level rise and increased incidents of extreme precipitation – are expected to diminish the size of the unsaturated treatment area and reduce the availability of O₂, both of which are important for the removal of contaminants. This may reduce the ability of soil-based OWTS to treat wastewater, especially in coastal zones with shallow water tables commonly found in southern New England.

Shallow narrow STAs are assumed to provide better wastewater renovation and may be more resilient to the effects of climate change than conventional STA. Conventional STAs receive wastewater from the septic tank, where infiltration occurs deeper in the soil profile. The shallow narrow STAs receive pre-treated wastewater from secondary treatment components that allow shallower dispersal of effluent compared to the conventional STA, providing a large volume of soil for treatment. Current understanding of the differences in performance among STA types is rudimentary, and their response to climate change is

unknown. I used replicated ($n = 3$) intact soil mesocosms to measure the performance of two shallow narrow STAs – shallow narrow drainfield (SND) and Geomat® (GEO) – and a conventional pipe and stone (P&S) STA, and their response to climate change.

I first evaluated the water quality functions of conventional and shallow narrow STAs under present climate conditions. Between 97.1 and 100% of BOD₅, fecal coliform bacteria (FCB) and total P were removed in all STA types. Total N removal averaged 12.0% for P&S, 4.8% for SND, and 5.4% for GEO. All STA types performed similarly for most water quality functions despite differences in carbon and O₂ content, input wastewater, dosing regimen, and placement of infiltrative surface within the soil profile.

I also examined the mechanisms of N removal within conventional and shallow narrow STAs using a ¹⁵N tracer. Nitrogen removal in the STA is attributed to N₂ production via heterotrophic denitrification, with little direct evidence to support this. Removal of N in the gas phase was attributable primarily to N₂, which had a flux $10^2 - 10^3$ times larger than N₂O in all STAs. The constraints imposed by differences in availability of electron donors and acceptors in different STAs pointed to autotrophic N removal processes (e.g. anaerobic ammonia oxidation, autotrophic denitrification) as playing an important role in N removal in addition to heterotrophic denitrification processes.

The impacts of climate change on the STAs were evaluated by raising the water table in the mesocosms 30 cm and increasing the soil temperature 5°C. Greater removal of BOD₅ was observed under climate change for all STA types. Release of FCB increased from <1 (present climate) to up to 20 CFU 100 mL⁻¹ under climate change, likely the result of lower attachment of bacteria in saturated soil and greater transport to groundwater. Climate change resulted in decreased total P removal, from 75-100% under present climate to 66-72%, possibly due to reduction of Fe and Mn oxides involved in the formation of insoluble P-metal complexes. Total N removal increased from 14.2% to 19% for conventional STA, but

decreased from 5.6-7.0% to $< 3.0\%$ for shallow narrow STAs under climate change. Higher BOD_5 removal in the latter may have lowered N removal by limiting carbon availability to microorganisms responsible for heterotrophic denitrification. Climate change is likely to affect contaminant removal in the STA, with the extent of effects depending on the contaminant and type of STA. To mitigate climate change impacts, I suggest that planners, regulators and OWTS designers investigate methods for carbon additions to the STA and reduce reliance on the soil by utilizing more effective and sustainable pre-treatment measures to reduce treatment variability.

ACKNOWLEDGEMENT

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PREFACE

This dissertation is presented in manuscript format in accordance with University of Rhode Island Graduate School Guidelines. There are five sections contained within this dissertation, an introduction, three chapters and conclusions. The first chapter is entitled “*Evaluation of Water Quality Functions of Conventional and Advanced Soil-Based Onsite Wastewater Treatment Systems*” and authored by J.A. Cooper, G.W. Loomis, D.V. Kalen, and J.A. Amador, and has been published at the Journal of Environmental Quality. The second chapter is entitled “*Nitrogen Transformations in Different Types of Soil Treatment Areas Receiving Domestic Wastewater*” and authored by J.A. Cooper, I. Morales, and J.A. Amador, and is in revision for publication in Ecological Engineering. The third chapter is entitled “*Hell and High Water: Diminished Septic System Performance Due to Climate Change*” and authored by J.A. Cooper, G.W. Loomis and J.A. Amador, is in preparation for submission to PLOS ONE.

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INTRODUCTION

Soil-based onsite wastewater treatment systems (OWTS) are the most common option for wastewater treatment in rural and unsewered watersheds of the U.S. due to their effectiveness and relative low cost (EPA, 2002). An estimated 1.5×10^{10} L of wastewater is processed every day by 26 million OWTS in the U.S. (EPA, 2002; U.S. Census Bureau, 1997). Onsite wastewater treatment systems are also widely employed in Canada, Europe, Australia, New Zealand and Japan, and are often the only technology available for wastewater treatment in developing countries. With a current world population of 7 billion (Census Bureau, 2014), most of which does not have access to publically owned wastewater treatment facilities, the issue of how to treat wastewater in rural and unsewered areas is an important public health and environmental problem at the global scale.

Onsite wastewater treatment systems have been identified as the third largest contributor to groundwater pollution (USEPA, 2002). Human excreta and urine contain a variety of contaminants long established as harmful to human and environmental health, including pathogenic bacteria and viruses, protozoan cysts, nematode eggs, and excess organic carbon (C) (measured as biochemical oxygen demand, BOD₅), nitrogen (N) and phosphorus (P).

Release of bacterial and viral pathogens to ground or surface water as a result of incomplete wastewater treatment pollutes drinking water, contaminates shellfish beds, and jeopardizes public health by contaminating recreational waters (USEPA, 2002). Many pathogenic microorganisms require relatively small doses to cause infection and induce illness in humans. For example, *E. coli* O157:H7, which produces

shiga toxin and can cause kidney failure and death, requires fewer than 10 cells to cause illness (USDA, 1992). Likewise, rotavirus, the leading cause of severe diarrhea worldwide, can cause illness or death in humans from ingestion of a single viral particle (Graham, 1987). The feces of an infected human will release 10^5 - 10^8 cells of *E. coli* O157:H7 (Feachem et al., 1980) or $10^8 - 10^{10}$ rotavirus particles (Gerba, 1996), making effective removal of pathogens from wastewater a public health priority.

Excess inputs of N to groundwater from poorly treated wastewater can also be detrimental to human health. High nitrate concentrations in drinking water can disrupt oxygen (O_2) binding to red blood cells (Shuval and Gruener, 1972), causing methemoglobinemia, also known as “blue baby syndrome”, in infants. In addition, Ward et al. (1996) has observed a correlation between elevated nitrate levels and incidence of Non-Hodgkins lymphoma.

Insufficiently treated wastewater can also damage aquatic ecosystems from excess inputs of BOD_5 and nutrients. Release of BOD_5 promotes microbial consumption of available O_2 and may cause hypoxia. High N and P levels in surface water can cause excess algal growth (eutrophication) of marine and freshwater ecosystems, respectively, that reduce dissolved O_2 when microorganisms decompose the algae (Howarth and Marino, 2006). Anoxia may cause fish and other O_2 dependent organisms to perish. Eutrophication of drinking water reservoirs is also a severe problem in many parts of the world, which leads to production of algal toxins and precursors of carcinogenic compounds that endanger public health and restrict access to drinking water (Palmstrom et al., 1988).

Onsite wastewater treatment systems play a vital role in the renovation of

residential wastewaters. They serve communities that lack the infrastructure and finances to implement large-scale wastewater treatment plants. They also help maintain water resources by providing groundwater recharge, an especially important feature in drought-stricken regions.

Despite being used by nearly 25% of the U.S. population, the effectiveness of OWTS at removing contaminants from wastewater is not monitored in most of the U.S. Additionally, since the initial, simple design of a septic system (septic tank, distribution box and soil treatment area), there has been little innovation of conventional OWTS – used by the majority of the U.S. population – and thus little improvement in their treatment capacity. Attempts to improve OWTS performance have met with various levels of success. For example, inclusion of aerobic secondary treatment units increase O_2 levels of wastewater before application to the soil, and result in better removal of organic carbon and pathogens (Loomis et al., 2001). Improved N removal is achieved by using proprietary technologies, such as aerobic treatment technologies and recirculating media filters, that oxidize N, with subsequent recirculation back to a low O_2 reactor, where microorganisms convert N into N_2 . However, these systems have had variable effectiveness, are expensive and have resulted in limited regional adoption by OWTS users.

The soil treatment area (STA; also known as a drainfield or leachfield) of an OWTS is an important component for removal of contaminants from wastewater, particularly in conventional systems. Treatment of septic tank effluent takes place as it percolates through the unsaturated portion of the soil profile, where moisture and O_2 levels are conducive to removal of pathogens and microbial and chemical processes

that reduce the concentration of contaminants. Pathogens in the STA are removed by predation, absorption, and filtration (McCray et al., 2009), BOD₅ is quickly consumed by carbon-limited soil microbial communities (Schimel and Schaeffer, 2012), and nutrients undergo biochemical transformations and/or retention in soil (Robertson, 2003). The extent of treatment in the STA depends, to a large extent, on the volume of unsaturated soil the wastewater must pass through, represented by the vertical separation between the infiltrative surface of the STA and the water table (Cogger et al., 1988; Anderson et al., 1994; Powelson and Gerba, 1994; Stevik et al., 2004). Because it relies on hydrologic, microbial and chemical processes, treatment of wastewater in the STA is sensitive to environmental perturbations (e.g. changes in pH, moisture, temperature, O₂, presence of toxins) that may reduce the treatment capacity.

Under our present climate conditions OWTS may be underperforming, and projected changes in climate conditions may be further detrimental to their treatment capacity. Sea level rise due to climate change will reduce the volume of unsaturated soil available for wastewater treatment in low-lying near shore coastal areas. Sea levels in the Northeastern U.S. are projected to rise 90-120 cm by 2100 (IPCC, 2013), resulting in higher water tables in coastal regions. Rhode Island has already experienced 20 cm of sea level rise since 1930 (RICCC, 2012). Precipitation events in the Northeastern U.S. are expected to increase in number and severity over the same time period (IPCC, 2013). The combined effects of higher water tables and increased precipitation will result in wetter soils. Wetter soils have been shown to increase survival of bacterial and viral pathogens (Campbell et al., 1976; Quanrud, 2003), may lead mobilization of P from reduction of metal bound P complexes (Robertson, 2003),

but may enhance removal of N by microbial reduction to N_2 by limiting O_2 diffusion. Additionally, the degree of soil moisture can either aid or hinder decomposition of organic carbon (Davidson et al., 2000), which will directly affect BOD_5 removal.

Elevated temperatures due to climate change will also effect OWTS contaminant treatment in the STA. The IPCC predicts atmospheric temperatures will increase 2-5°C in the next 100 years in the Northeastern U.S (IPCC, 2013). Warmer conditions have been shown to increase bacterial and viral pathogen mortality (Gerba et al., 1975; Nasser et al., 2002). However, higher temperatures will also reduce O_2 solubility and promote microbial O_2 consumption, resulting in less O_2 available for aerobic treatment processes, likely diminishing P removal. Warmer temperature will increase microbial activity and may increase the rate of BOD_5 consumption, potentially limiting heterotrophic processes such as denitrification.

The combined effects of temperature and sea level rise are expected to be detrimental to overall contaminant removal in the STA under climate change conditions. Because 40% of the U.S. population resides in coastal communities (NOAA, 2011), this will likely impact coastal communities that rely on OWTS for wastewater renovation, as well as systems in shallow water table areas that were installed decades ago, and where a rising water table has slowly reduced vertical separation distances.

Different types of STAs may react differently to climate change. The STA in a conventional OWTS is located deep in the soil profile where infiltration of septic tank effluent (STE) into coarser textured soil with larger pores reduces the likelihood of hydraulic failure due to clogging. A shallow narrow STA receives effluent that has

undergone secondary treatment (i.e. aeration, oxidation, and nitrification), resulting in higher dissolved O_2 levels, and reduced levels of BOD_5 and particulates, prior to dispersal to an infiltrative surface that is placed higher in the soil profile than a conventional STA. Because the advanced treated wastewater has low levels of BOD_5 , a biomat doesn't develop in shallow narrow STAs, unlike in conventional STAs. In addition, shallow narrow STA designs incorporate frequent timed-dosing of small volumes of wastewater, preventing prolonged periods of soil saturation, which are common in a conventional STA.

Shallow narrow STAs are generally assumed to provide better wastewater renovation, however, these assumption had not been tested experimentally. I studied the water quality functions of a conventional pipe and stone (P&S) STA in comparison to two types of shallow narrow STAs – a pressurized shallow narrow drainfield (SND), and Geomat (GEO), a proprietary variation of the SND. The three STA types were evaluated in triplicate using intact soil mesocosms under current climate conditions for one year. The result of this study were published in the Journal of Environmental Quality.

The results of the above study raised questions about the mechanisms of N removal within the STA. Denitrification is generally credited with N removal in the STA, with losses occurring as gaseous N_2 and N_2O . However, losses of N from other mechanistic pathways have not been considered, although these are recognized in other disciplines, nor have N gas fluxes from STAs been measured. To determine how N is removed in the STA, aqueous and gaseous N species were measured within the soil profile of the conventional and shallow narrow STAs following introduction of a

¹⁵N tracer. This manuscript is currently under revision for publication in Ecological Engineering.

Onsite wastewater treatment systems are hypothesized to have diminished performance under climate change. However, this has not been tested experimentally, nor has the resilience of differing STA types been studied under climate change conditions. To assess the impacts of climate change on OWTS, we measured water quality functions of conventional and shallow narrow STAs under present climate conditions (20°C and water tables set at technology regulatory specifications) in comparison to climate change conditions (25°C and water tables elevated 30 cm). The results of this study has been submitted for publication to the PLOS ONE.

**MANUSCRIPT – I: EVALUATION OF WATER QUALITY FUNCTIONS OF
CONVENTIONAL AND ADVANCED SOIL-BASED ONSITE WASTEWATER
TREATMENT SYSTEMS**

Published in the Journal of Environmental Quality, May 2015

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ABSTRACT

Shallow narrow drainfields are assumed to provide better wastewater renovation than conventional drainfields, and are employed for protection of surface and ground water. To test this assumption, we evaluated the water quality functions of two advanced onsite wastewater treatment systems (OWTS) drainfields – shallow narrow (SND) and Geomat® (GEO) – and a conventional pipe and stone (P&S) drainfield over 12 months using replicated ($n = 3$) intact soil mesocosms. The SND and GEO mesocosms received effluent from a single-pass sand filter, whereas the P&S received septic tank effluent. Between 97.1 and 100% of BOD₅, fecal coliform bacteria and total phosphorus (P) were removed in all drainfield types. Total nitrogen (N) removal averaged 12.0% for P&S, 4.8% for SND, and 5.4% for GEO. A mass balance analysis accounted for 95.1% (SND), 94.1% (GEO) and 87.6% (P&S) of N inputs. When the whole treatment train (excluding the septic tank) is considered, advanced systems – including sand filter pre-treatment and SND or GEO soil-based treatment – removed 99.8–99.9% of BOD₅, 100% of fecal coliform bacteria and P, and 26.0 – 27.0% of N. In contrast, the conventional system removed 99.4% of BOD₅, 100% of fecal coliform bacteria and P, but only 12.0% of N. All drainfield types performed similarly for most water quality functions despite differences in placement within the soil profile. However, inclusion of the pre-treatment step in advanced system treatment trains results in better N removal than in conventional treatment systems, despite higher drainfield N removal rates in the latter.

INTRODUCTION

Onsite wastewater treatment systems (OWTS) include a soil-based treatment component – also known as a drainfield or soil treatment area (STA) – that aids in wastewater renovation, such as removal of BOD₅, nutrients, and pathogenic microorganism from wastewater. Advanced OWTS are used in areas where conventional systems are not considered adequate to protect public and environmental health. These systems are used in coastal and shallow water table areas sensitive to nutrients and pathogens, where the level of renovation by a conventional, pipe and stone drainfield may be insufficient. Nitrogen (N) is of particular concern because it can cause eutrophication of coastal ecosystems (Howarth and Marino, 2006), and N is poorly removed in conventional drainfields (USEPA, 2002).

A conventional drainfield receives septic tank effluent (STE), whereas the STA of advanced systems receives effluent from an advanced treatment system, such as a single-pass sand filter (SFE), with reduced concentrations of biochemical oxygen demand (BOD₅) and total suspended solids (TSS) (Loomis, 2001). In advanced systems, dosing of treated wastewater to soil is generally to a shallow-placed drainfield infiltrative surface at 15–30 cm below the ground surface, whereas in conventional systems the drainfield infiltrative surface is placed ~60 cm below the ground surface (Tyler et al., 1977). The placement of shallow narrow drainfields provides a larger volume of unsaturated soil for treatment that is thought to allow more O₂ diffusion (Birkham, 2007), as well as enhanced filtration when finer textured soil is found higher in the soil profile (Romero, 1970). A larger unsaturated vertical separation between the infiltration area and the water table has been shown to be

important for attenuation of bacteria, viruses, BOD₅, and phosphorus (P) (Cogger et al., 1988; Anderson et al., 1994; Powelson and Gerba, 1994; Stevik et al., 2004).

Dosing systems for shallow narrow drainfields have different configurations to improve oxygenation of wastewater by increasing O₂ diffusion into the aqueous phase before infiltration. In systems that include an advanced treatment step, the drainfield receives wastewater (SFE) that has a higher concentration of dissolved oxygen (DO) than that in STE. By contrast, a conventional drainfield is gravity fed with anoxic wastewater to a layer of crushed and washed stone that serves as storage during periods of large volume use, with little opportunity for aeration. Timed-dosing controls for shallow narrow drainfields provide consistent moisture levels, preventing the extended saturation periods experienced by conventional drainfields (Rubin and Janna, 2006). Together, these design variations are expected to result in differences in the extent and mechanisms of wastewater renovation.

Examination of the literature shows that most shallow drainfield studies have been conducted with STE dosed to the drainfield. Phosphorus removal is nearly complete in shallow drainfields (e.g. Stewart and Reneau, 1988; Gill et al., 2009), as is removal of fecal coliform bacteria (Ijzerman et al., 1992, Ijzerman et al., 1993). In contrast, N removal is variable with values ranging from 18 to 75% (Bunnell et al., 1999; Gill et al., 2009; Siegrist et al., 2014). The performance of these drainfields when dosed with a pre-treated effluent (SFE) has been evaluated to a limited extent. Holden et al. (2004a, 2004b) found complete removal of P and 18–44 % N removal in several shallow narrow drainfields receiving SFE. The water quality function of shallow drainfields has not been well studied in comparison to conventional

drainfields; however Bunnell et al. (1999) found similar N removal rates for shallow and conventional drainfields receiving STE. This suggests that these drainfield types may differ less than is widely assumed.

Nearly 40% of the U.S. population is concentrated in coastal communities (NOAA, 2011), where nutrient and pathogen contamination are of particular concern. Nationally ~ 25% of the population relies on OWTS for wastewater renovation (U.S. Census Bureau, 2011). Although both advanced and conventional drainfield systems are currently used to renovate wastewater, quantitative data that allow for comparisons of their wastewater renovation capacities are lacking. To address this information gap, we evaluated the wastewater renovation functions of two types of shallow narrow drainfields and a pipe and stone (P&S) drainfield using replicated ($n = 3$) intact soil core mesocosms. Using mesocosms allowed us to control for variables that may preclude direct comparisons of results, such as differences in temperature, soil properties, exogenous inputs of nutrients, pathogens and water, and wastewater composition.

MATERIALS AND METHODS

Description of mesocosms. Nine intact soil cores were collected in PVC pipes (7600 PVC type 1; 152-cm tall x 15-cm-diam.), and excavated in October 2012 from a grassy area along a 5-m long trench in Kingston, Rhode Island, USA. The soil at the site is a Bridgehampton silt loam (Coarse-silty, mixed, active, mesic Typic Dystrudepts) (Table 1.1). Triplicate soil cores were engineered to represent one of three drainfield types in the laboratory: (i) pressurized shallow narrow drainfield

(SND), (ii) GeoMat[®] (GEO), and (iii) pipe and stone (P&S) (Supplemental Fig. S1.1). Mesocosms were prepared by initially filling with tap water from the bottom, and dosed with tap water at steady flow rates for 75 days. They were allowed to drain by gravity for two days before introduction of wastewater.

The soil infiltrative area was established at 20 cm below the ground surface (Ap1 horizon; Table 1.1) for SND, at 25 cm (Ap1/Ap2 horizon) for GEO, and at 84 cm (2C2 horizon) for P&S. The water table was controlled using a hanging water column (Supplemental Fig. S1.1a) and was set at 102 cm below the infiltrative surface for SND and GEO, and at 56 cm for P&S. This established the water table at 122-140 cm from the ground surface for all drainfield types, and reproduced expected field conditions.

The SND delivery device (Supplemental Fig. S1.1b) was constructed from a halved, 10-cm-diam. PVC pipe placed to form a dome over the infiltrative surface, with Impolene tubing (Imperial Eastman, Baltimore, MD) inserted through the top to produce sheet flow over the inside dome surface. The GEO delivery device (Supplemental Fig. S1.1b) was constructed from a perforated, 2.5-cm-diam. PVC pipe, fitted with a plastic diffuser plate, and placed over a 2-cm thick fused plastic filament mesh and geotextile filter fabric in contact with the infiltrative surface. The P&S delivery device was built from 10-cm-diam. PVC pipe with two 1-cm-diam. holes drilled at 22.5° from vertical at either side to allow wastewater dispersal (Supplemental Fig. S1.1b) and placed between two layers of washed stone with a 1.75 to 5.0 cm size range.

Ceramic-tipped probes (YSI, Yellow Springs, OH) were used to measure soil temperature at depths of 0, 15, 30, 45, 60 and 75 cm below the infiltrative surface for SND and GEO, and at 0 and 15 cm below the infiltrative surface for P&S (Supplemental Fig. S1.1a). iButton sensors (DS1921G, Maxim Integrated, San Jose, CA) were used to measure hourly soil surface temperatures. Soil EC-5 moisture probes and Em5b data loggers (Decagon Devices, Pullman, WA) were used to measure soil moisture every 15 minutes at depths of 15, 30, 45, 60 and 75 cm below the infiltrative surface for SND and GEO, and at 15 and 30 cm below the infiltrative surface for P&S (Supplemental Fig. S1.1a).

Gas sampling ports (1-mL, 5-cm-long, slotted plastic syringes wrapped in plastic screen mesh) were placed at 15 cm above and 15, 30, 45, 60 and 75 cm below the infiltrative surface of SND and GEO, and at 15 and 30 cm above and below the infiltrative surface for P&S (Supplemental Fig. S1.1a). To replicate the atmosphere of a drainfield, soil-filled plastic 18.9-L containers (36-cm tall, 30-cm diam.) were connected to the bottom of the infiltrative area of each mesocosm (Supplemental Fig. S1.1a).

Wastewater sources. Wastewater was collected weekly from a two-person private residence in South Kingstown, RI, USA. The system treatment train consisted of a septic tank with a 5,678 L capacity and a median STE flow of 314 L d⁻¹ (range: 102 – 700 L d⁻¹), with subsequent passage through a 15-year-old single-pass sand filter (surface area of 21 m² and designed loading rate of 81.6 L m⁻² d⁻¹) before dispersal to a drainfield. Field DO was determined with a model 55 DO meter (YSI) or by the

Winkler method (LaMotte, Chestertown, MD) (APHA, 1998). Field wastewater temperature was determined with an alcohol thermometer.

Mesocosm dosing. Wastewater was stored in sealed plastic containers at 4°C in the dark after collection (to maintain its integrity) before dispensing small volumes to thermoelectric chilled (Coolworks, San Rafael, CA) plastic holding containers maintained at 4–16°C for dosing.

Septic tank effluent for the P&S mesocosm was stored in a plastic container sealed from the atmosphere and connected to a N₂-filled, 2-L Tedlar bag (DuPont, Pascagoula, MS). The mesocosms were dosed at a rate of 400 mL d⁻¹ (22.6 L m⁻² d⁻¹), applied in two, 200-mL doses every 12 h over 1.5 h using a peristaltic MiniPump (Thomas Scientific, Swedesboro, NJ) and tygon and Impolene tubing

Sand filter effluent for SND and GEO was stored in a plastic container that had a small opening to the atmosphere. The mesocosms were dosed at a rate of 2 L d⁻¹ (113 L m⁻² d⁻¹) applied in 48, 42-mL doses, every 30 min over 15 min with a multichannel peristaltic pump (IPC-N-24 V2.03, Ismatec, Wertheim, Germany) using tygon, Pharmed BPT (Cole Palmer, Vernon Hills, IL) and Impolene tubing. Dosing rates were based on regulations governing OWTS design in the state of Rhode Island (RIDEM, 2013).

Mesocosm moisture and temperature conditions. The mean soil temperature for all drainfield types was maintained at 20.0 ± 0.7°C in the infiltrative area, with values lower in the soil profile deviating no more than 1°C from the mean.

The moisture content at 15-cm below the infiltrative area was maintained at $0.15 \text{ m}^3 \text{ m}^{-3}$ for SND and $0.12 \text{ m}^3 \text{ m}^{-3}$ for GEO, with lower values at greater depths ($0.02\text{--}0.08 \text{ m}^3 \text{ m}^{-3}$) and little variation observed over time. The P&S drainfield experienced a peak in soil moisture at 15 cm ($0.07 \text{ m}^3 \text{ m}^{-3}$) every 12 h, with moisture content slowly dropping between doses to $0.03 \text{ m}^3 \text{ m}^{-3}$. The soil moisture content at 30 cm below the P&S infiltrative area ranged from $0.0\text{--}0.02 \text{ m}^3 \text{ m}^{-3}$.

Soil analysis. Soil morphology was determined in the field, following the protocol in Schoeneberger et al. (2012). Bulk density was determined using the core method (Blake, 1965), particle size distribution was measured using the hydrometer method (Bouyoucos, 1962), cation exchange capacity using the method of Chapman (1965), and organic matter content according to Schulte and Hopkins (1996). Soil electrical conductivity (EC) and pH were measured using a 1:5 soil to water ratio, with EC measured using a model 06-662-61 probe (Control Company, Friendswood, TX) and pH determined with an Ultrabasic 10 pH meter (Denver Instruments, Bohemia, NY). *In situ* mesocosm soil pH was measured with an IQ 150 pH meter (Spectrum Technologies, Aurora, IL) and ISFET stainless steel microprobe (HACH, Loveland, CO).

Water analyses. Water outputs from the mesocosms were collected at the level of the water table in autoclaved, N_2 purged 1-L Nalgene bottles fitted with an airlock. Samples for NH_4 , NO_3 , PO_4 and SO_4 analysis were filtered through a $0.45\text{-}\mu\text{m}$ -pore-size membrane and stored frozen in plastic vials. The pH of NH_4 samples was adjusted to 2 with sulfuric acid before freezing. Samples for total N (TN) and total P (TP) analysis were not filtered before freezing.

Water pH was determined using an Ultrabasic 10 pH meter (Denver Instruments). Five-day biochemical oxygen demand (BOD₅) was determined using Oxitop BOD pressure sensor heads (WTW, College Station, TX) at $20 \pm 3^{\circ}\text{C}$. This method yields results that are nearly identical to those using the oxygen sensor and iodometric titration methods (Roppola et al., 2007). Fecal coliform bacteria and *E. coli* were enumerated by the membrane filtration method (APHA, 1998). Total suspended solids (TSS) concentrations were determined gravimetrically (APHA, 1998). Electrical conductivity was measured using a model 06-662-61 probe (Control Company). Samples for TN and TP analysis were digested using the persulfate oxidation method (APHA, 1998). Colorimetric methods were used to determine NO₃ (Doane and Horwath, 2003), NH₄ (Weatherburn, 1967), and PO₄ (Murphy and Riley, 1962) concentrations using a Bio-Tek microplate reader (Powerwave 340, Winooski, VT). Sulfate was measured turbidimetrically (APHA, 1998) using a model UV160U UV-visible spectrophotometer (Shimadzu Corp., Columbia, MD). Limits of detection were 0.05 mg L⁻¹ for TN, TP, NH₄, NO₃, PO₄; 0.1 mg L⁻¹ for SO₄; and 1.0 mg L⁻¹ for BOD₅ and TSS.

Gas sampling and analysis. Gas samples were collected with an air-tight, 20-mL syringe and either dispensed into pre-evacuated 20-mL glass vials fitted with red rubber septa (13 × 20 mm, Wheaton, Millville, NJ) for CH₄ and N₂O analysis, or injected immediately after sampling to a flow-through cell connected to an O₂ probe (model O2-BTA, Vernier, Beaverton, OR). Vials were stored inverted and submerged in water. Gases were analyzed using a Shimadzu Gas Chromatograph-2014 Greenhouse Gas Analyzer (Kyoto, Japan) fitted with a flame ionization detector (FID)

for CH₄ and an electron capture detector (ECD) for N₂O analysis. Instrument temperatures were 100°C (injection), 80°C (column), 250°C (FID) and 325°C (ECD). The carrier gas (ultra-pure N₂) flow rate was 25 mL min⁻¹.

Dissolved gas concentrations were determined as described by Jahangir (2012), with injection of 1-mL of STE or SFE, or 5-mL of output water into a 42-mL glass bottle, and gas masses calculated as described in USEPA (2004).

Gas fluxes from the top of the mesocosm were measured with a gas-tight PVC cap fitted with a silicone O-ring. Samples were collected at 0, 15 and 30 min after capping. Gas flux values (g min⁻¹ m⁻²) were calculated using the equation:

$$\Phi = G \times \left(\frac{P}{RT} \right) \times \left(\frac{V_h}{V_{smp}} \right) \times \frac{M}{A} \quad [\text{Eq. 1}]$$

where Φ = gas flux (g min⁻¹ m⁻²), G = measured gas production rate (L min⁻¹), P = atmospheric pressure (1 atm), R = ideal gas law constant (1.08205 L-atm mol⁻¹K⁻¹), T = temperature (K), V_h = volume of headspace (L), V_{smp} = volume of sample (L), M = molecular weight (g mol⁻¹), and A = cross-sectional area of mesocosm (m²).

Mass balance calculations. An N mass balance for the mesocosms was calculated using the equation:

$$TN_{in} + N_2O_{diss\ in} = TN_{out} + N_2O_{diss\ out} + N_2O_{flux\ out} \quad [\text{Eq. 2}]$$

where TN_{in} = input total N, $N_2O_{diss\ in}$ = input dissolved N₂O, TN_{out} = output total N, $N_2O_{diss\ out}$ = output dissolved N₂O, and $N_2O_{flux\ out}$ = N₂O soil gas flux.

Statistical analyses. Data for BOD₅ and TN removal, output SO₄, and soil pore N₂O and CH₄ concentrations in the infiltrative area were tested for normality with

a Shapiro-Wilk test, and differences evaluated using a Kruskal-Wallis One-Way ANOVA on Ranks. Means separation was accomplished using Dunn's method for TN, Tukey's Test for N₂O and CH₄ concentrations in the infiltrative area, and output SO₄ concentrations. Data for O₂ concentrations in the infiltrative area and in output DO were tested for normality with a Shapiro-Wilk test, and differences evaluated using a One-Way ANOVA, with means separation accomplished using the Holm-Sidak method. All statistical tests were evaluated at $p \leq 0.05$.

RESULTS AND DISCUSSION

Water Constituents

The physical, chemical, and microbiological characteristics of STE and SFE (Table 1.2) were within the range of those reported by others (Siegrist, 2001; Loomis et al., 2001; Potts et al., 2004).

pH. The pH of output water declined steadily for the first four months, subsequently reaching a stable value of 3.6 for all three drainfield types (Fig. 1.1), despite differences in input pH (6.4 for STE and 4.7 for SFE; Table 2). Others have observed higher pH in output water, closer to neutral or slightly acidic (Siegrist et al., 2014; Stewart and Reneau, 1988). These results suggest that a common mechanism controlled the pH of output water, independent of drainfield and wastewater type. Microbial oxidation of NH₄ and reduced S compounds, observed in all three drainfield types, is the most likely source of acidity. The buffering of output water at pH 3.6 in all three drainfields was likely due to hydrolysis and precipitation of aluminum (Al) hydroxide minerals released in this acidic soil (Jackson, 1963). Binding of H⁺ to soil

surfaces releases Al^{3+} , hydrolyzing water and binding the released OH^- to precipitate $\text{Al}(\text{OH})_x$ species, leading to buffering at $\text{pH} \sim 3.5$ (Jackson, 1963).

Fecal coliform bacteria. We observed complete removal of fecal coliform bacteria in all drainfield types (Supplemental Figure S1.2). Others have reported similar results, with complete removal of fecal coliform bacteria in 30 cm (Atoyan et al., 2007) and 60–90 cm (Anderson et al., 1994) of soil, within the range of soil depths in our experiment. Removal of fecal coliform bacteria for all drainfield types was the same among drainfield types despite differences in soil texture at the infiltrative surface and soil depth (Table 1.1), suggesting that other factors may be responsible for bacterial removal. For example, the acidic pH of soil (Supplemental Fig. S1.3) and output water (Fig. 1.1), coupled with Al toxicity at low pH, may have contributed in a similar manner to the attenuation of fecal coliform bacteria in all drainfield types.

Phosphorus. We observed complete removal of TP in all drainfield types (Supplemental Figure S1.4) despite coarser texture soil below the infiltrative area of P&S (Table 1.1). Removal of P from wastewater is expected to be higher for finer textured soils (Brandes et al., 1975), such as those found below the infiltrative area of SND and GEO, which have a higher proportion of reactive soil particle surfaces. In a field study of P retention, Robertson (2003) found the highest P removal in acidic septic plumes ($\text{pH} 4.9\text{--}5.5$) in soil containing high Al. That study also demonstrated that the amount of Al/Fe oxides and low pH were more important for P removal than soil texture, which ranged from silt to coarse sand (Robertson, 2003). The low buffering capacity and low pH of our soil (Table 1, Supplemental Fig. S1.3) can result in dissolution of gibbsite and release of Al^{3+} ions that irreversibly retain P in the soil

by precipitation of Al-phosphate minerals (Robertson, 2003). Removal of P can also occur by formation of insoluble oxides with Fe (Robertson, 2003). The Bridgehampton silt loam used in our study typically contains 3.5 – 3.7% Fe oxides and 3.1 – 4.6% Al, with higher values observed in the A and B horizons (Bell and Shearin, 1967). This, in conjunction with the acidic pH of soil and output water, point to P retention by reaction with Al and Fe oxides as a reasonable mechanism for all three drainfield types.

BOD₅. Removal of BOD₅ was 99.3, 97.1 and 98.1% for P&S, SND and GEO, respectively, with no significant differences among treatments (Supplemental Figure S1.5). Anderson et al. (1994) also observed complete BOD₅ removal from STE within 60 cm of soil depth in a mesocosm study. Soil microbial communities are carbon (C)-limited (Schimel and Schaeffer, 2012). Organic C in BOD₅ is thus expected to be quickly depleted with passage of wastewater through the soil, despite differences in texture, soil depth, and dissolved O₂ (Fig. 1.2) among drainfield types.

Nitrogen. Within the soil drainfield, differences in TN concentration between input and output water were positive – indicating net N removal relative to inputs – in 40 out of 47 weeks for P&S (removal of TN from STE), compared to 28 weeks of net TN removal from SFE over the same period for SND and GEO (Fig. 1.3). The average removal rate for TN over the course of a year in P&S was 12.0%, significantly higher than for SND (4.8%) and GEO (5.4%). Removal of N from a conventional drainfield can range from 0–30% (USEPA, 2002), whereas the extent of N removal in shallow narrow drainfields has not been reported in the literature. Nitrification was observed in all drainfield types, as indicated by increases in NO₃ concentrations and lower NH₄

concentrations in output water, and an associated drop in pH relative to wastewater inputs (Fig. 1.1). Although ammonia-oxidizing bacteria that can function under acidic soil conditions have been identified (Prosser and Nicol, 2012), given the sensitivity of most nitrifying bacteria to acidic conditions and the low pH of output water and soil (Supplemental Fig. S1.3), nitrification may have occurred in the first few centimeters below the infiltrative surface. Alternatively, ammonia-oxidizing archaea that are capable of functioning in acidic conditions (Yao et al., 2011), may have also contributed to NO_3 production. We observed a high proportion of organic N (14–16%) in output water for all drainfield types, possibly originating from unprocessed organic N in inputs and/or *in situ* microbial production. Organic N is not generally considered as an N input to groundwater from soil-based treatment systems. However, its presence in relatively high concentrations in water from drainfields raises questions about its potential impact on N dynamics in receiving waters.

Sulfate. We observed similar dynamics of SO_4 in output water from all drainfield types (Fig. 1.1). Output water SO_4 concentrations dropped sharply for all three drainfield types during the first three months of the experiment, subsequently increasing to, and remaining at, initial concentrations. The initial decline in SO_4 concentrations in output water may have resulted from the establishment of acidic conditions in soil and water (Supplemental Fig. S1.3 and Fig. 1.1) – resulting from ammonia and sulfur oxidation – that created an unfavorable environment for sulfate-reducing bacteria, and favored sulfur-oxidizing chemolithotrophic bacteria, which can function at an acidic pH (Germida and Janzen, 1993). A plot of SO_4 vs. pH (Supplemental Fig. S1.6) in output water shows that concentrations of SO_4 were

directly proportional to pH during the first three months of the experiment, but showed little variation with pH subsequently. Adsorption of SO_4 , favored at lower pH, may have also contributed to the initial decline in output SO_4 concentrations; however, PO_4 may compete for adsorption sites (Kamprath et al., 1956). Throughout the experiment, SO_4 concentrations in output water in P&S were significantly lower than in SND and GEO, indicating that extent and mechanisms of S transformations differed.

Gases

Oxygen. Dissolved oxygen increased in all drainfield types after passage through soil (Fig. 1.2). The average DO concentration over the course of a year in output water from SND and GEO was $2.6 \text{ mg O}_2 \text{ L}^{-1}$, significantly higher than in P&S output water ($1.7 \text{ mg O}_2 \text{ L}^{-1}$). Dynamics of DO in output water were similar for all three drainfield types, with DO concentrations declining to relatively constant values after the first 6 months of operation, possibly indicating development of a biofilm at the infiltrative surface that reduced the permeability of soil to water and gases (Beal, 2006).

Gas phase concentrations of O_2 in the infiltrative area of P&S were significantly lower than for SND and GEO (Supplemental Fig. S1.7). Soil pore O_2 levels compared well to those reported in Kristiansen (1980) after steady state was reached, and were below ambient atmospheric levels at all depths for all drainfield types, indicating net consumption of O_2 in the soil profile. However, higher DO in output water than input water suggests that diffusion of O_2 from the gas phase to aqueous phase in the soil was greater than microbial O_2 consumption.

Methane. Dissolved CH₄ concentrations in input wastewater to P&S were 300 µg C L⁻¹, decreasing to 0.3 µg C L⁻¹ in output water. Dissolved CH₄ in input wastewater to SND and GEO was 4 µg C L⁻¹, and also decreased in output water to 0.3 – 0.5 µg C L⁻¹ (Fig. 1.2). The CH₄ concentration in the infiltrative area was significantly higher in P&S, over an order of magnitude higher than in SND and GEO, which were similar to atmospheric concentrations (Supplemental Fig. S1.7). Methane concentrations in the soil were indistinguishable from atmospheric concentrations in SND and GEO throughout the soil profile, whereas in P&S they were 10× above those in the atmosphere (Supplemental Fig. S1.7), and similar to those reported by Kristiansen (1980) for a sand filter receiving STE.

Losses of CH₄ from the mesocosms were not due to direct gas phase losses to the atmosphere (Supplemental Fig. S1.8), since no net flux of CH₄ was detected in any drainfield type. Rather, losses of CH₄ likely took place via methane oxidation to CO₂, which can be aerobic or anaerobic (Kightley et al., 1995). While aerobic CH₄ oxidation requires O₂, anaerobic CH₄ oxidation can be coupled with SO₄ reduction, and has been observed in marine sediments (Hoehler et al., 1994). The latter may explain lower output SO₄ concentrations in P&S, and a higher soil pH at 30-cm below the infiltrative area in P&S (Supplemental Fig. S1.3).

Nitrous oxide. Average dissolved N₂O concentrations in input wastewater to P&S were 9.2 µg N L⁻¹, increasing to 20 µg N L⁻¹ in output water. By contrast, dissolved N₂O in input wastewater to SND and GEO was 200 µg N L⁻¹, decreasing to 30 µg N L⁻¹ in output water (Fig. 1.2). The small amount of N₂O present in STE may have formed from nitrification and/or denitrification in the septic tank, whereas

dissolved N₂O in SFE was likely from nitrification in the sand filter. Others have shown that, in the absence of O₂, little N₂O is produced by denitrification, whereas the amount of N₂O produced via nitrification and denitrification increases with O₂ levels (Wrage, 2001; Wunderlin et al., 2012; Richard et al., 2014). The flux of N₂O was significantly higher from SND (63 $\mu\text{g N m}^{-2} \text{ h}^{-1}$) and GEO (55 $\mu\text{g N m}^{-2} \text{ h}^{-1}$) than P&S (3 $\mu\text{g N m}^{-2} \text{ h}^{-1}$). The higher N₂O flux from SND and GEO likely results from a combination of outgassing of dissolved N₂O from input water and *in situ* microbial production. Because dissolved N₂O concentrations increased in P&S with passage through the soil (Fig. 1.2), the flux of N₂O is probably from *in situ* microbial production (Supplemental Fig. S1.8).

Concentrations of N₂O in the headspace of the infiltrative area were above atmospheric concentrations in P&S, but significantly lower than SND and GEO. The concentration of N₂O in the soil in SND and GEO was 20 \times higher than in P&S, indicating higher *in situ* production (Supplemental Fig. S1.7).

Nitrogen Mass Balance

We calculated a mass balance for N entering and exiting the drainfields to help quantify loss pathways (Fig. 1.4). In P&S, outputs (514 $\text{g N m}^{-2} \text{ yr}^{-1}$) of N accounted for 87.6% of inputs (588 $\text{g N m}^{-2} \text{ yr}^{-1}$) to the drainfield, with 12.4 % (74 $\text{g N m}^{-2} \text{ yr}^{-1}$) unaccounted for. Loss of N occurred mainly as dissolved N species, comprised of NO₃ (83%), organic N (16%) and NH₄ (1%). Nitrous oxide in gas and dissolved phases accounted for 0.04 % of N outputs, suggesting N₂O production was not a major loss pathway in P&S.

Nitrogen was better accounted for in SND and GEO, with outputs (2194 and 2170 g N m⁻² yr⁻¹) accounting for 95.1 and 94.1% of inputs (2306 g N m⁻² yr⁻¹), respectively. Loss of N occurred mainly as dissolved N species, comprised of NO₃ (84–85%), organic N (14–15%) and NH₄ (<1%). Nitrous oxide in the gas and dissolved phase accounted for 0.08% of N loss in SND and GEO, indicating this was not an important pathway for net N loss in either drainfield type.

A small fraction of the unaccounted N in all three drainfield types is N stored in microbial biomass, which must have accumulated during the course of the experiment. A much larger fraction of the missing N is likely to be N₂ from denitrification. Denitrification requires an organic C-to-N ratio of 1.2:1 (Bitton, 2005), and anaerobic conditions that can be found in anaerobic microsites (Sextone, 1985), both of which are more likely to be met in P&S (C:N = 1.0:1). In contrast, wastewater inputs to SND and GEO are carbon limited (C:N = 0.07:1), which may restrict the extent of denitrification, and account for lower N removal in SND and GEO. Denitrification processes that do not require an organic C source may also be active in the mesocosms. For example, *Thiobacillus denitrificans* can reduce NO₃ to N₂, oxidizing reduced S compounds under anaerobic conditions instead of organic C (Roberston and Kuenen, 1991; Kanter et al., 1998), which could contribute to N removal in all drainfield types. This process has been observed under acidic conditions in soil (Germida et al., 1991). High concentrations of reduced S compounds in wastewater (Devai and DeLaune., 1999) and the production of SO₄ in the mesocosms (Fig. 1.1) lend support to this explanation.

Whole System Analysis

We used mesocosm and field data to compare the performance of whole conventional and advanced system, exclusive of septic tank treatment. Evaluation of the advanced systems included pre-treatment (single-pass sand filter) and soil-based treatment (SND or GEO drainfield), and these were compared to the conventional soil-based treatment (P&S). Using a measured average flow rate of 342 L d^{-1} , yearly inputs of contaminants are estimated to be 32.3kg BOD₅, 1.1×10^{12} CFU fecal coliform bacteria, 9.0 kg N, and 1.5 kg P (Fig. 1.5). Based on differences in concentration between STE and SFE (Table 1.2), we estimate that the single-pass sand filter removed 93% of BOD₅, 99% of fecal coliform bacteria, 22% of N, and 31% of P from STE inputs. When treatment by SND and GEO drainfields are included, the advanced treatment trains removed 99.8–99.9% of BOD₅, 26–27% of N, and 100% of fecal coliform bacteria and P from STE inputs. In contrast, the conventional P&S system removed 99.4% BOD₅, 12.0% of N, and 100% fecal coliform bacteria and P (Fig. 1.5). Inclusion of a sand filter treatment step improves N removal in the treatment train, even though this is not considered an N-removal technology in most jurisdictions. Vegetation, which was not grown in our mesocosms, could account for an additional ~2% N-removal in the SND and GEO drainfields (Holden et al., 2004a). Utilizing an N-removal advanced treatment technology that is approved to meet 19 mg L^{-1} effluent standards, combined with a shallow placed drainfield with an established grass cover, should help improve N reduction.

Our results show that the P&S, SND and GEO drainfields are equally effective for removal of BOD₅, fecal coliform bacteria, and total P. Furthermore, similar

mechanisms for water quality renovation appear to be at work in conventional and advanced OWTS, despite differences in placement in the soil profile, physical and chemical properties of the receiving soil, and separation distance from the water table. Dynamics of sulfate and pH were similar in all three drainfield types, suggesting similar process govern changes in their concentration. In contrast, N removal differed among the drainfield types, with 12.0% for P&S, 4.8% for SND and 5.4% for GEO. When the whole treatment train (except the septic tank) is considered, advanced systems that include sand filter pre-treatment and soil-based treatment exceeded the N removal capabilities of a conventional system. Our experimental design included the use of intact soil cores, replicated drainfield types, and wastewater inputs from the same source that experienced identical environmental conditions. Although conditions in the field may diverge from those in the laboratory, our experiment allowed us to make direct comparisons among drainfield types. Our results suggest that quantitative information regarding the wastewater renovation capacity of different drainfield types, an understanding of the mechanisms involved in renovation, and evaluation of their performance in the context of whole treatment systems are necessary to optimize system selection.

Acknowledgements

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TABLES

Table 1.1 Select morphological, physical and chemical properties of the soil used in drainfield mesocosms. Values for physical and chemical properties are means ($n = 7$) \pm SD Measurements of pH, electrical conductance (EC) and cation exchange capacity (CEC) were made on composite samples.

Horizon	Depth	Color	Texture	Particle size distribution			Structure	Bulk density	Porosity	Organic matter	pH	EC	CEC
	cm			Sand	Silt	Clay							
				%	%	%		g cm^{-3}	%	g kg^{-1}		μS	$\text{mEq } 100 \text{ g}^{-1}$
A _{p1} A _{p2}	0-31	brown	silt loam	72 \pm 13	21 \pm 12	10.2 \pm 0.6	weak granular to subangular blocky	1.08 \pm 0.06	59 \pm 2	0.5 \pm 0.03	4.9	32.7	3.9 \pm 0.1
B _w	31-44	yellowish brown	silt loam	74 \pm 13	18 \pm 13	8.1 \pm 1.2	weak med. subangular blocky	1.27 \pm 0.09	52 \pm 3	0.26 \pm 0.05	4.9	20.6	2.4 \pm 0.4
2B _w	44-58	light olive brown	gravelly loamy sand				weak med. subangular blocky						
2C1	58-70	light olive brown	v. gravelly coarse sand; 40% gravel	96 \pm 1	1.6 \pm 1.1	2.8 \pm 0.1	structureless single grain; loose	1.69 \pm 0.08	36 \pm 3	0.05 \pm 0.003	4.5	1.6	0.44 \pm 0.12
2C2	70-96	light yellowish brown	v. gravelly coarse sand; 45% gravel	94 \pm 3	3.6 \pm 3.4	2.7 \pm 0.1	structureless single grain; loose	1.61 \pm 0.06	39 \pm 2	0.06 \pm 0.02	4.4	2.7	0.48 \pm 0.15
2C3	96-130	pale yellow	coarse sand				structureless single grain; loose						

Table 1.2 Characteristics of septic tank effluent (STE) and sand filter effluent (SFE) used in our study (n = 26 – 49).

Property	STE		SFE	
	Median	Range	Median	Range
pH	6.4	5.9 – 7.3	4.7	3.2 – 6.1
Dissolved O ₂ , mg L ⁻¹	0.0	0.0 – 0.4	2.5	1.2 – 4.1
BOD ₅ , mg L ⁻¹	260	120 – 410	19	0 – 80
Total suspended solids, mg L ⁻¹	41	18 – 89	5.0	0.0 – 30
Electrical conductivity, µS	770	550 – 920	560	360 – 750
Fecal coliform bacteria, CFU 100 mL ⁻¹	3.6×10^5	3.0×10^4 – 4.5×10^6	3.0×10^2	6.0×10^0 – 3.9×10^4
<i>E. coli</i> CFU 100 mL ⁻¹	3.4×10^5	1.0×10^4 – 4.4×10^6	9.2×10^1	0 – 3.9×10^4
Total N, mg L ⁻¹	72	42 – 95	54	29 – 88
NH ₄ -N, mg L ⁻¹	56	40 – 74	14	6.0 – 34
NO ₃ -N, mg L ⁻¹	0.1	0.0 – 0.9	30	10 – 58
Total P, mg L ⁻¹	11	6.8 – 17	7.8	3.8 – 13
PO ₄ -P, mg L ⁻¹	6.4	3.3 – 7.9	4.3	2.7 – 6.2
SO ₄ -S, mg L ⁻¹	0.8	0.2 – 7.2	9.3	4.2 – 28.8
Collection temperature, °C	16	5 – 22	15	4 – 21

FIGURES

Figure 1.1 Concentrations of pH, NH_4 , NO_3 , and SO_4 in inputs and outputs for pipe and stone (P&S), shallow narrow drainfield (SND), and GeoMat® (GEO) drainfield mesocosms. Values are means ($n = 3$) over 12 months. Error bars represent one calculated SD for each mean.

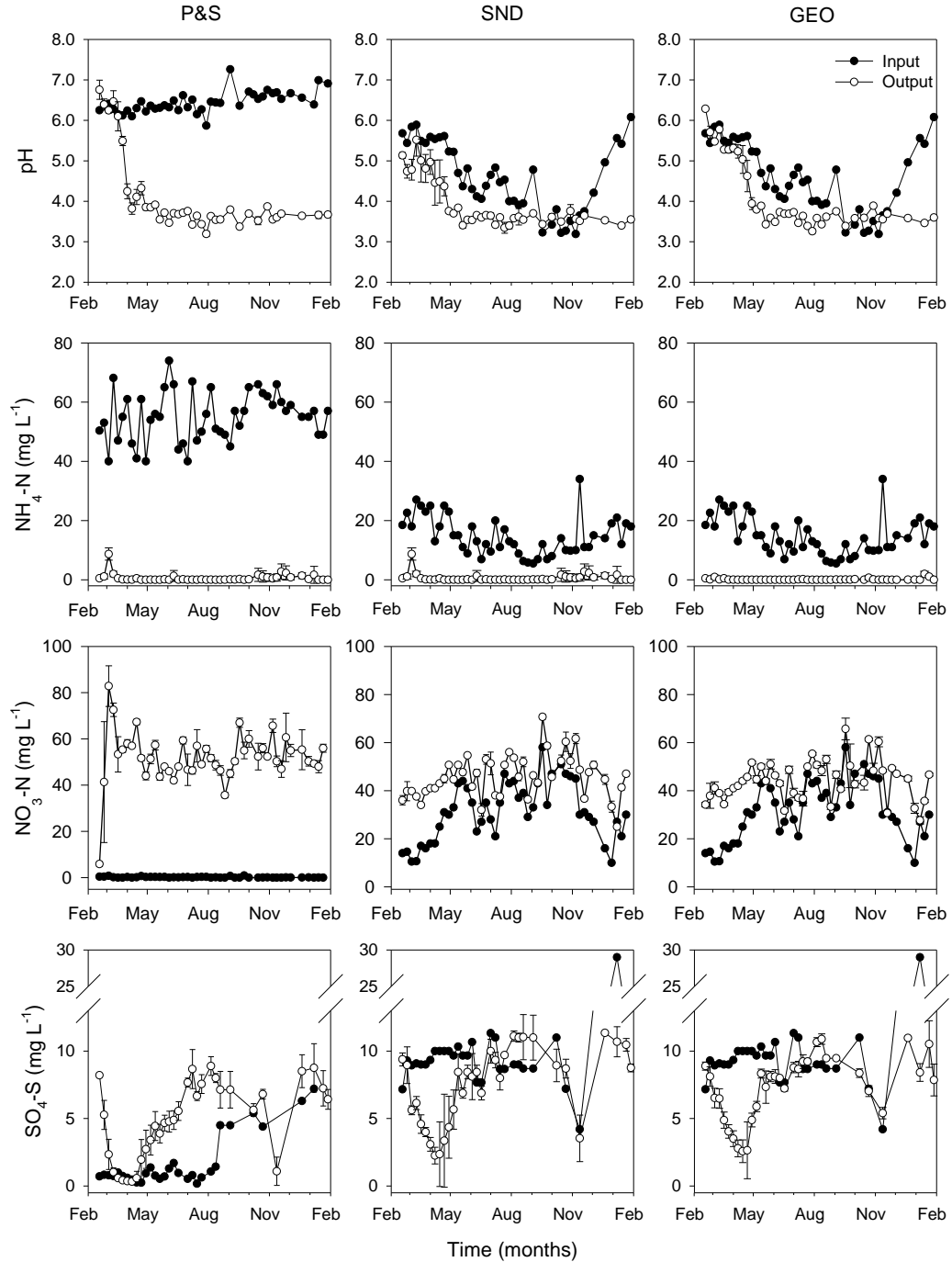


Figure 1.2 Dissolved O_2 , CH_4 and N_2O concentrations in input and output water for pipe and stone (P&S), shallow narrow drainfield (SND), and GeoMat[®] (GEO) mesocosms. Values are means ($n = 3$) measured between Aug. 2013 and Feb. 2014. Error bars represent one calculated SD for each mean. Note log scale for CH_4 and N_2O , and linear scale for O_2 .

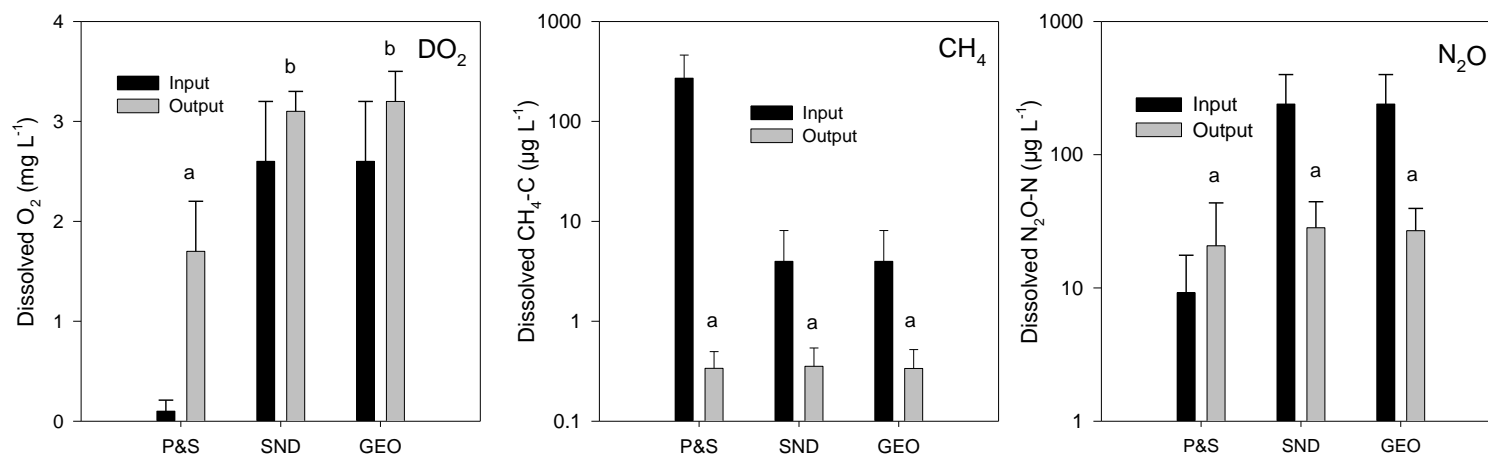


Figure 1.3 Net nitrogen removal in pipe and stone (P&S), shallow narrow drainfield (SND), and GeoMat® (GEO) mesocosms. Values are means ($n = 3$) over 12 months. Error bars represent one calculated SD for each mean.

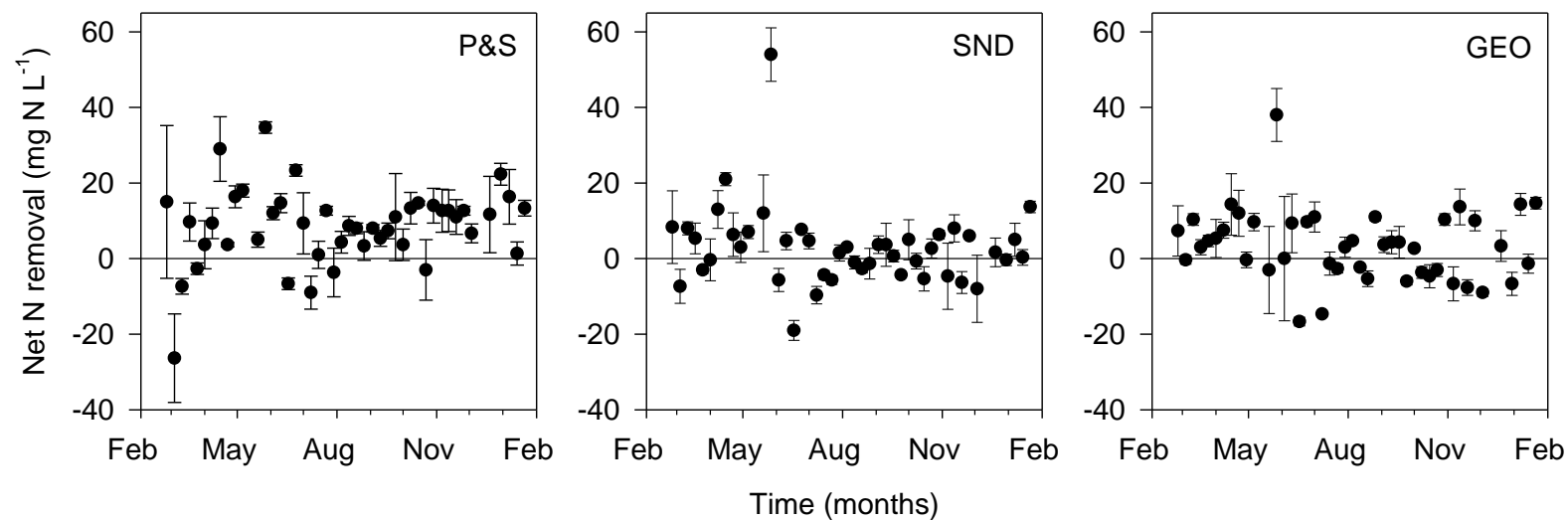


Figure 1.4 Inputs and outputs of N for pipe and stone (P&S), shallow narrow drainfield (SND), and GeoMat[®] (GEO) mesocosms over the course of a year. Values in parenthesis are $\text{g m}^{-2} \text{yr}^{-1}$.

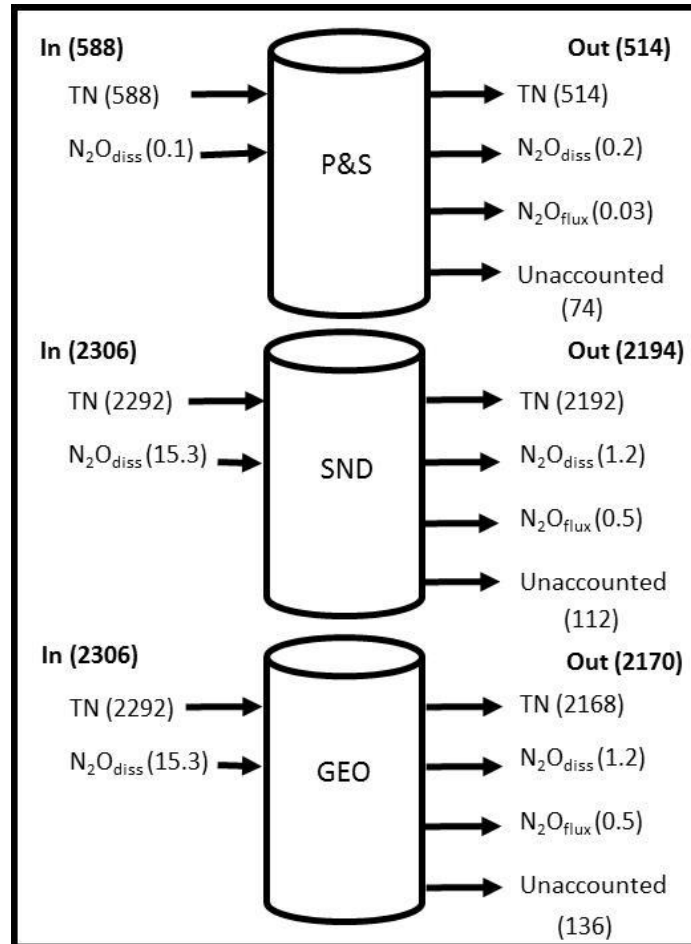
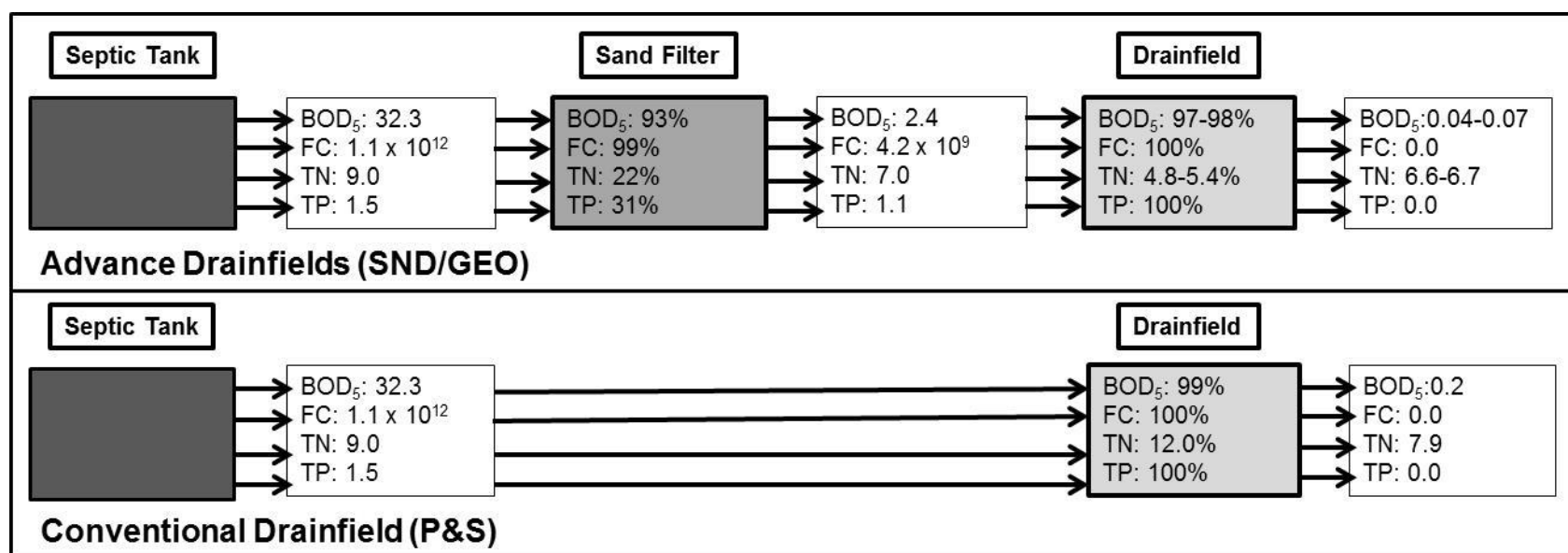
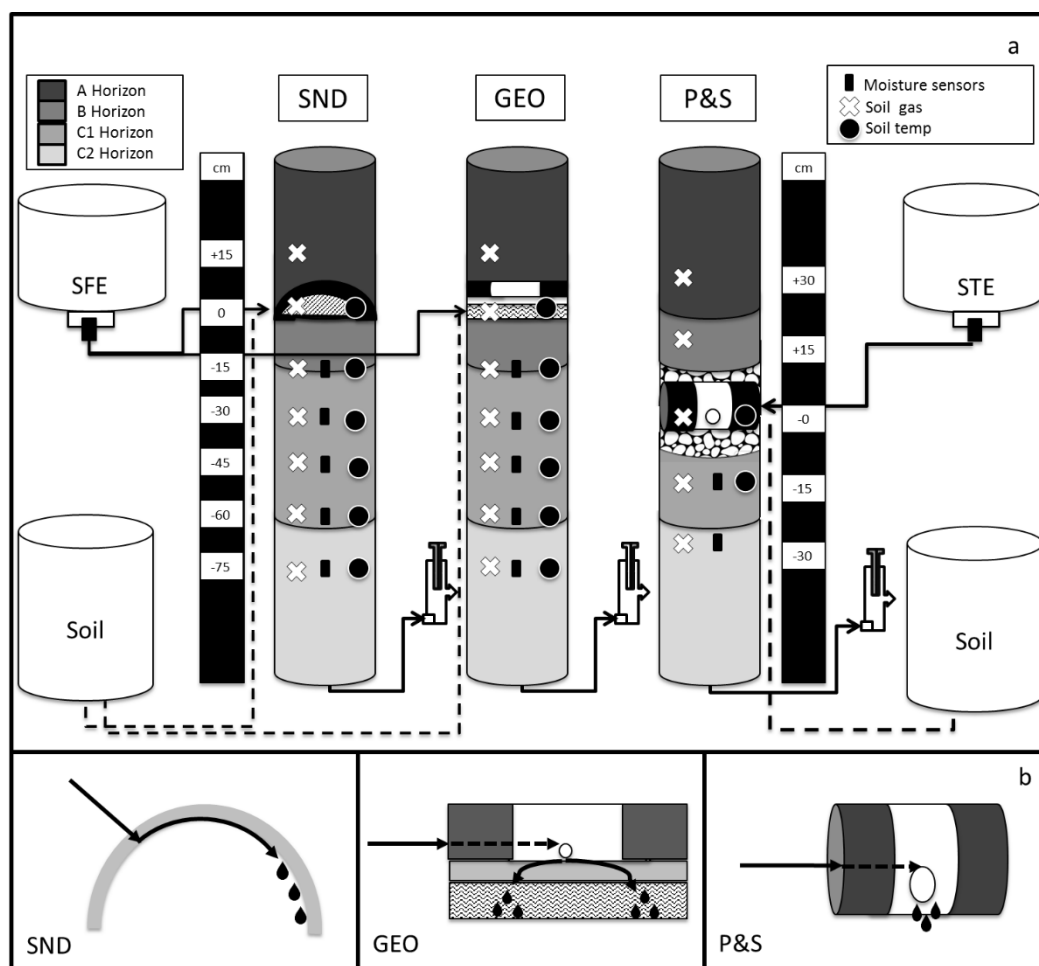


Figure 1.5 Estimates of field-scale mass loading from septic tank, sand filter and soil-based treatment for an advanced system with a shallow narrow drainfield (SND) or GeoMat® (GEO) drainfield, and for a conventional system with a pipe and stone (P&S) drainfield. Removal values (%) are for the previous step in the treatment train. Units are kg yr⁻¹ except for fecal coliform bacteria (FC), which are CFU yr⁻¹.

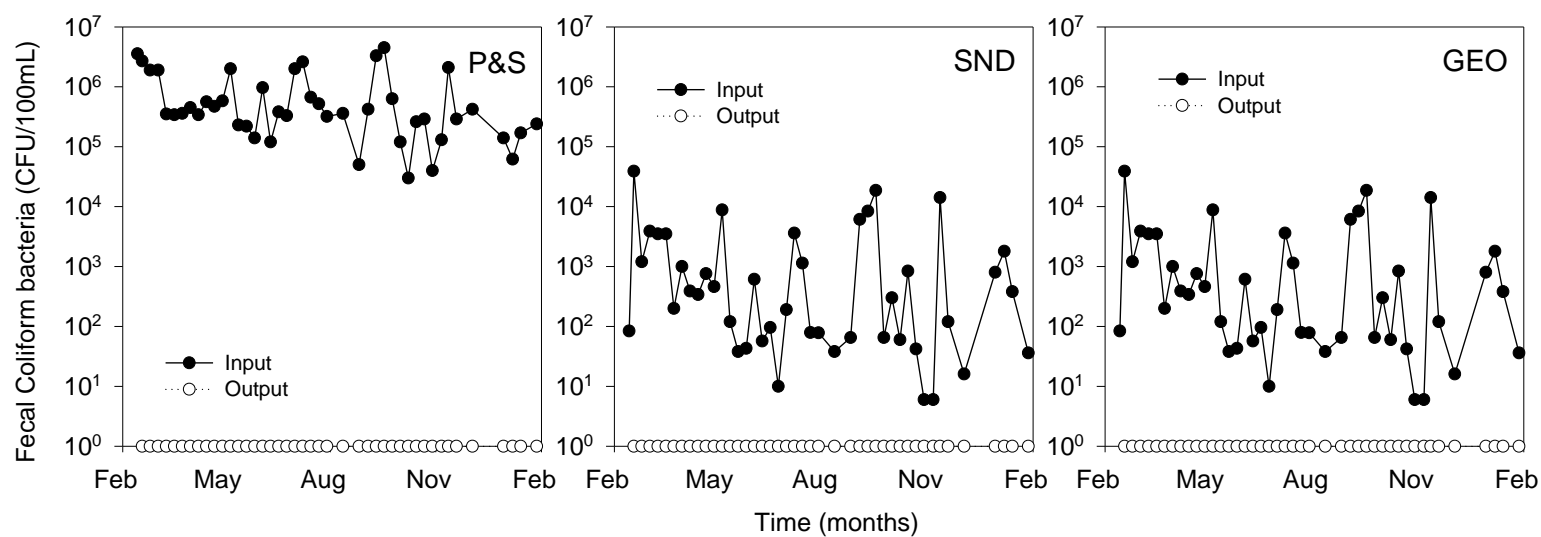


SUPPLEMENTAL MATERIAL

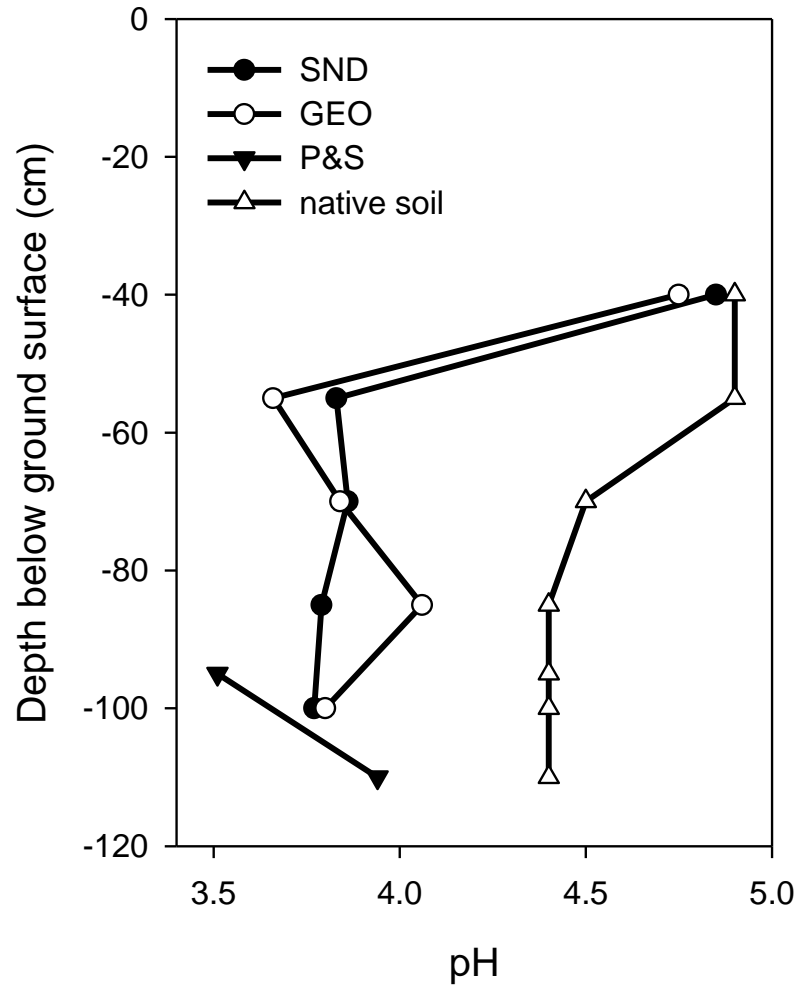
Supplemental Fig. S1.1 (A) Schematic diagram of soil mesocosms representing a shallow narrow drainfield (SND), GeoMat® (GEO), and pipe and stone (P&S) drainfield. The wastewater input to SND and GEO was sand filter effluent (SFE), whereas the P&S received septic tank effluent (STE). The approximate location of soil horizons, ports for gas sampling, and moisture and temperature probes are indicated. Water exits the mesocosms through a hanging water column device used to adjust the height of the water table. The atmosphere in the infiltrative area is connected to a 30-cm soil column. (B) Detailed schematic diagram of the SND, GEO and P&S delivery devices. Diagrams are not to scale.



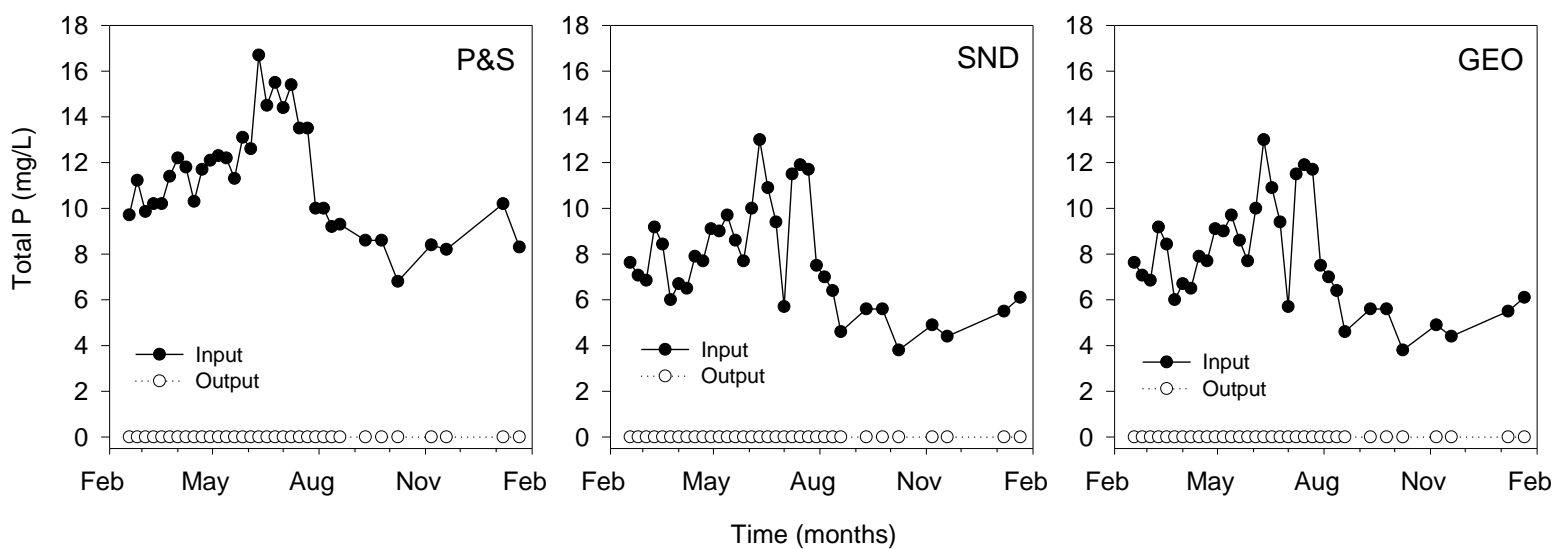
Supplemental Fig. S1.2 Concentration of fecal coliform bacteria in inputs and outputs for the pipe and stone (P&S), shallow narrow drainfield (SND), and GeoMat® (GEO) drainfield mesocosms.



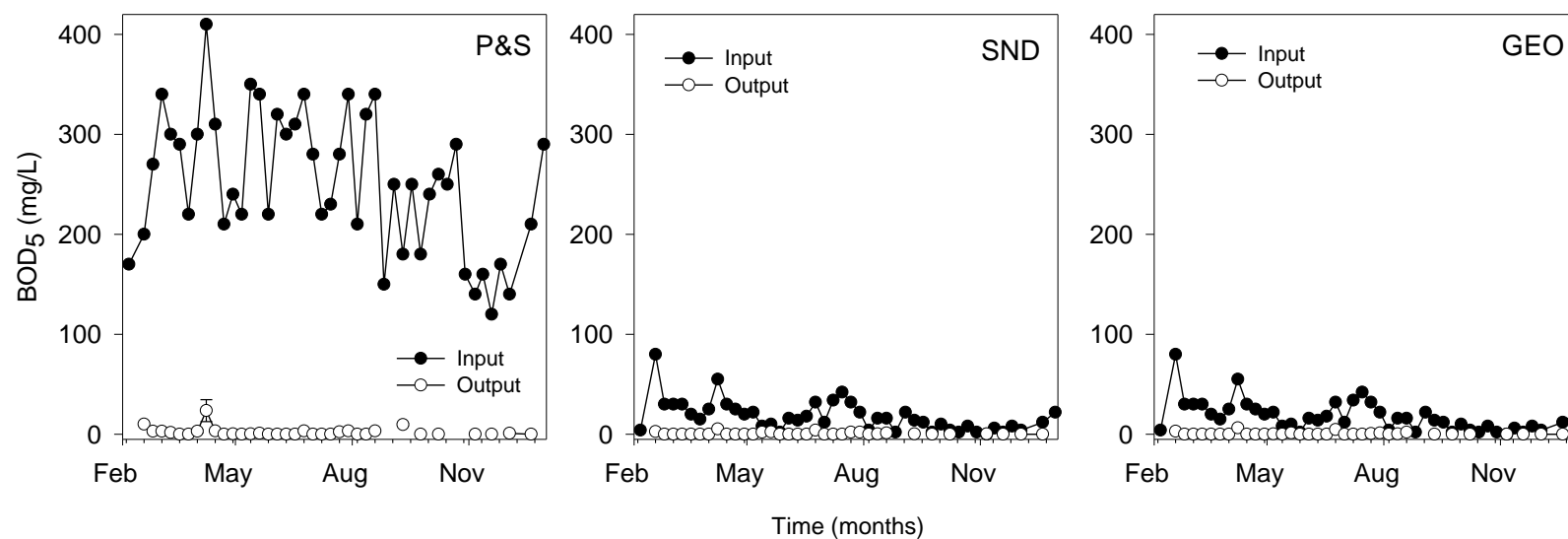
Supplemental Fig. S1.3 Depth profile of soil pH for shallow narrow drainfield (SND), GeoMat® (GEO), and pipe and stone (P&S) mesocosms after operation for 12 months for a single mesocosm, and of soil prior to wastewater dosing.



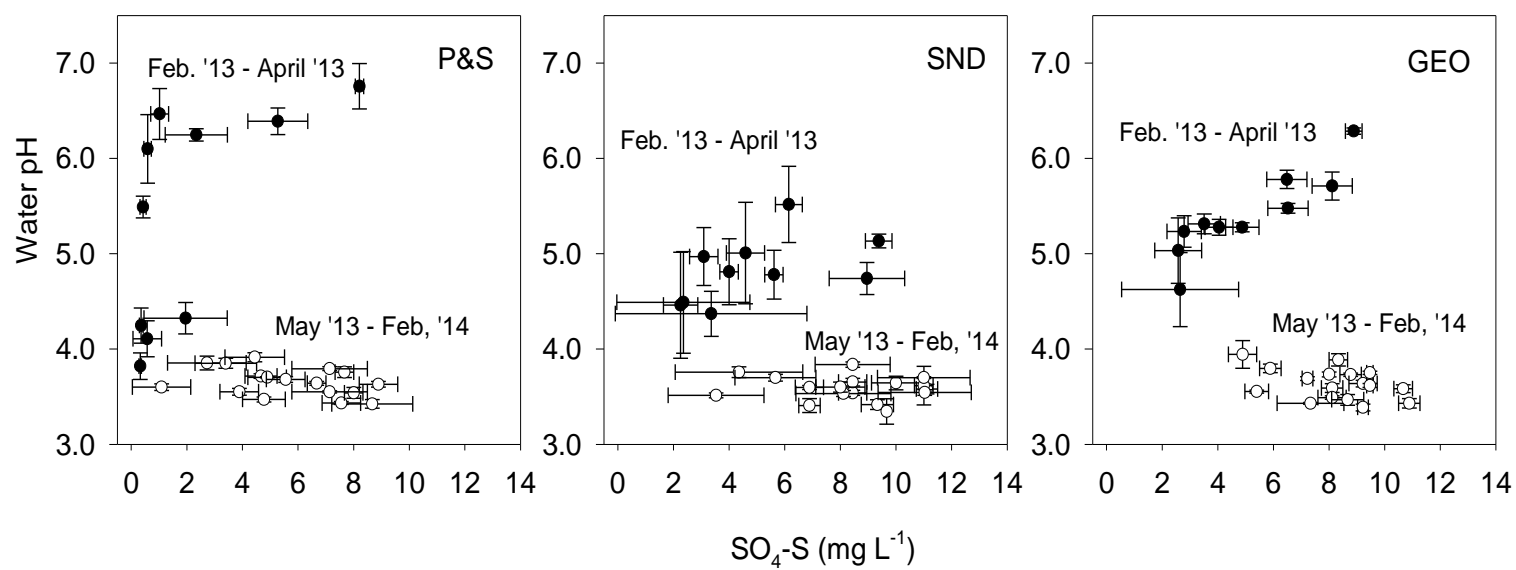
Supplemental Fig. S1.4 Concentration of total phosphorus (TP) in inputs and outputs for the pipe and stone (P&S), shallow narrow drainfield (SND), and GeoMat® (GEO) drainfield mesocosms.



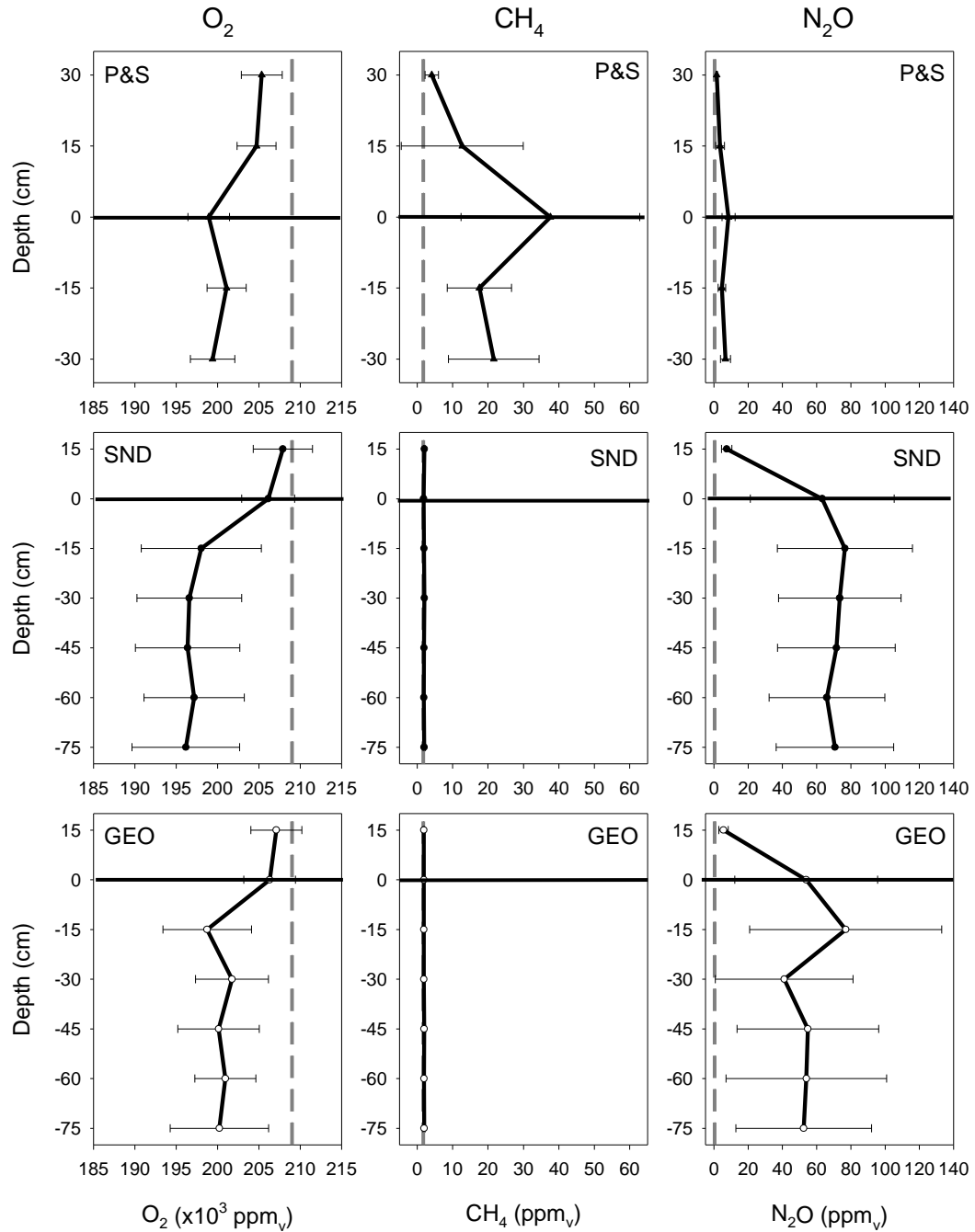
Supplemental Fig. S1.5 Concentration of BOD₅ in inputs and outputs for the pipe and stone (P&S), shallow narrow drainfield (SND), and GeoMat® (GEO) drainfield mesocosms. Output values are shown as the detection limit of 1 CFU mL⁻¹.



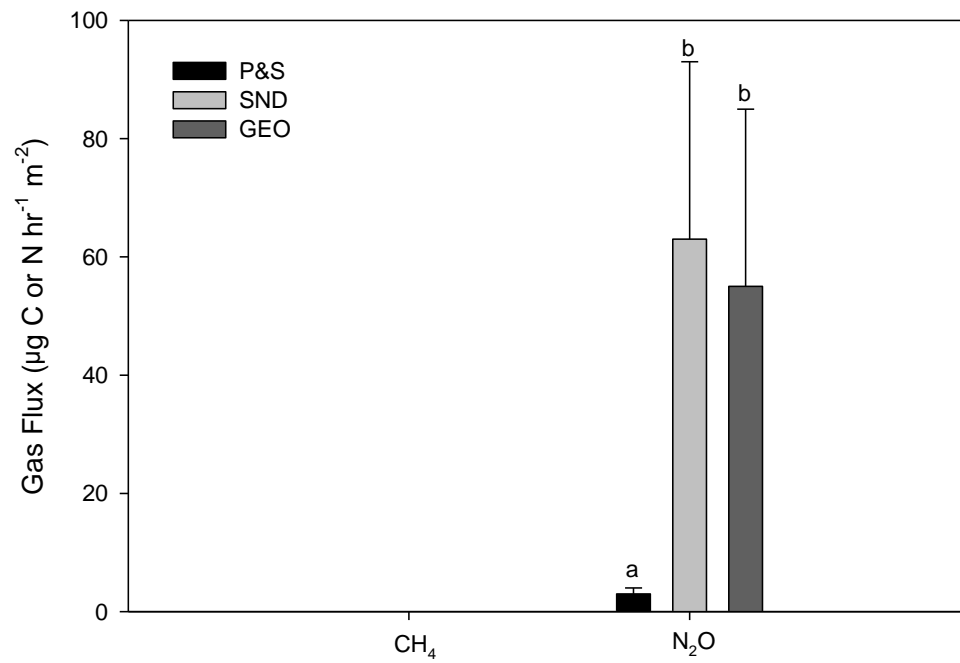
Supplemental Fig. S1.6 Relationship between SO_4 concentration and pH of output water from pipe and stone (P&S), shallow narrow drainfield (SND), and GeoMat[®] (GEO) mesocosms. Values are means ($n = 3$). Error bars represent one calculated SD for each mean.



Supplemental Fig. S1.7 Depth profiles of O₂, CH₄ and N₂O in pipe and stone (P&S), shallow narrow drainfield (SND), and GeoMat® (GEO) and mesocosms. Values are means for a single mesocosm from each drainfield type, measured between Aug. 2013 and Feb. 2014. Error bars represent one calculated SD for each mean. Dashed line represents ambient atmospheric gas concentration. Solid line at 0 cm represents position of the infiltrative surface.



Supplemental Fig. S1.8 Flux of CH₄ and N₂O in pipe and stone (P&S), shallow narrow drainfield (SND) and GeoMat® (GEO) mesocosms. Values are means for a single mesocosm from each drainfield type, measured between Aug. 2013 and Feb. 2014. Error bars represent one calculated SD for each mean.



**MANUSCRIPT – II: NITROGEN TRANSFORMATIONS IN DIFFERENT
TYPES OF SOIL TREATMENT AREAS RECEIVING DOMESTIC
WASTEWATER**

In review at the Journal of Ecological Engineering

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ABSTRACT

Removal of N within the soil treatment area (STA) of onsite wastewater treatment systems is attributed to heterotrophic denitrification, with N lost to the atmosphere as N_2 . However, the evidence supporting heterotrophic denitrification as the sole process for N removal is scant. We used $^{15}\text{NH}_4^+$ to follow N transformations in intact soil mesocosms representing a conventional STA receiving anoxic, C-rich wastewater, and two shallow-placed STAs receiving partially oxygenated, low-C wastewater. Nitrogen losses in the gas phase took place almost exclusively as $^{15}\text{N}_2$ in all STA types. We observed $10^2 - 10^3$ times higher flux of N_2 than N_2O in all STAs, as well as net production of $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ near the infiltrative surface and at greater depths in the soil profile. *In situ* net production of $^{15}\text{NH}_4^+$ suggested internal recycling of inorganic N in all STAs. The constraints imposed by differences in availability of electron donors and acceptors and soil physicochemical parameters in different STAs, point to autotrophic N removal processes (e.g. anaerobic ammonia oxidation, autotrophic denitrification) as playing an important role in N removal. These are more likely to contribute to N losses in C-limited, shallow-placed STAs, as well as deeper within the profile of all STAs, where C availability is limited. Our results suggest that N transformations and loss processes are more complex than currently thought.

1. INTRODUCTION

The soil treatment area (STA; also referred to as drainfield or leachfield) of onsite wastewater treatment systems (OWTS; also referred to as septic systems) is optimized for wastewater infiltration and removal of pathogens, but not N removal. Increases in the size and density of populations served by OWTS have caused higher N concentrations in receiving ground and surface water (Valiela et al., 1992). Elevated N inputs to aquatic environments cause eutrophication of coastal and fresh water ecosystems (Howarth and Marino, 2006), and negatively impacts human health (Oakley et al., 2012).

Nitrogen removal does take place in the STA, with rates ranging from 0 to 51% (Bunnell et al., 1999; Siegrist et al., 2014; USEPA, 2002). However, the processes involved are poorly understood. Heterotrophic denitrification, which produces N_2 and N_2O , is generally considered to be responsible for N removal (Bradshaw and Radcliffe, 2013; Bunnell et al., 1999; Crites and Tchobanoglous, 1998), and is thought to occur near the infiltrative surface. Conditions that support N removal by heterotrophic denitrification are likely to prevail in conventional STAs, which receive periodic inputs of anoxic effluent with high organic C and NH_4^+ levels, followed by oxic periods during which NO_3^- can be produced. Heterotrophic denitrification is less likely in a shallow-placed, or advanced, STAs because wastewater inputs have higher dissolved oxygen (DO) levels and much lower organic C levels due to rapid biodegradation in pre-treatment steps.

Processes other than heterotrophic denitrification can result in N removal in gaseous forms, including nitrification (N_2O), anaerobic ammonia oxidation (N_2), and autotrophic denitrification (N_2 and N_2O) (Table 2.1). Attributing all N removal in the STA to heterotrophic denitrification may be an over simplification of the processes involved. Autotrophic denitrifiers can reduce NO_3^- to N_2 using reduced S compounds (Kanter et al., 1998; Robertson and Kuenen, 1991) or CH_4 (Islas-Lima et al., 2003) as an electron donor, both of which are present in wastewater. Production of N_2 via anaerobic oxidation of NH_4^+ with either NO_2^- (anammox) (Robertson et al., 2012; Strous et al., 1997) or Fe^{3+} (feammox) (Yang et al., 2012) is also possible, with NO_2^- produced *in situ* and Fe^{3+} produced in soil. Loss of N as N_2O can also occur from autotrophic nitrification (Wrage et al., 2001). These processes all have different requirements in terms of C, electron donors, electron acceptors and redox conditions that can be met in different niches with the STA.

An improved understanding of N transformations in the STA, particularly those that result in gaseous N losses, can help optimize N removal. In a previous study, we observed 12.0% N removal from a conventional STA receiving septic tank effluent, and 4.8 – 5.4% N removal from two advanced STAs receiving wastewater that had been treated in a single-pass sand filter (Cooper et al., 2015). In the present study, we examined the transformations of N in these STAs using wastewater amended with $^{15}\text{NH}_4^+$. We measured aqueous ($^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$) and gaseous ($^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$) species over time and within the soil profile of the STA. In addition, we quantified variables that may affect N transformation, including BOD_5 , pH, SO_4^{2-} , CH_4 , CO_2 , O_2 , and water-filled pore space (WFPS).

2. MATERIALS AND METHODS

2.1 Experimental setup

We used intact soil mesocosms (152-cm tall \times 15-cm-dia.) to represent three STA types: (i) conventional pipe and stone (P&S), (ii) shallow narrow drainfield (SND) and (iii) Geomat® (GEO), a SND variation (Supplemental Fig. S2.1). The P&S was dosed with 200 mL of septic tank effluent (STE) every 12 h over 1.5 h, corresponding to 400 mL d⁻¹ (22.6 L m⁻² d⁻¹). The SND and GEO received wastewater that had passed through a single-pass sand filter (SFE). They were dosed with 22.5 mL SFE every 30 min over 15 min, corresponding to 2000 mL d⁻¹ (113 L m⁻² d⁻¹). Septic tank effluent and SFE were collected weekly from the same treatment train at a residence in Charlestown, RI, USA. Characteristics of wastewater inputs can be found in Table 2.2. Further details of the experimental design, sampling, and analytical methods can be found in Cooper et al. (2015).

2.2 ¹⁵N tracer experiment

The experiment was conducted between 7 January and 8 February 2014, after the soil mesocosms had been in continuous operation for 52 weeks. ¹⁵N-labeled ammonium chloride (¹⁵NH₄Cl, ~98+ At. %, Isotec Chemical Co., Miamisburg, OH) was added to either STE (for P&S) or SFE (for SND and GEO). We added 420 µg, 482 µg, and 391 µg of ¹⁵N to P&S, SND and GEO, respectively, resulting in final concentrations of 2.1, 3.7, and 3.1 mg ¹⁵N L⁻¹. Wastewater amended with ¹⁵N was delivered to the P&S drainfield in a single, 200-mL dose pumped over 1.5 h. Delivery of ¹⁵N-amended wastewater to the SND and GEO drainfields was achieved by dosing with 126 mL (three, 42-mL doses every 30 min for 15 min) over 1.5 h.

2.3 Analysis of ^{15}N in aqueous and gas phase.

Water samples for $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ analyses were diffused using to the acidified disk diffusion method (Khan et al., 1998) at 20°C using 10 mL samples. Diffusates and gases were analyzed at the University of California-Davis Stable Isotope Facility. Diffusates were analyzed with a continuous flow isotope ratio mass spectrometer (PDZEuropa, Northwich, UK) after combustion of samples at 1000°C to convert samples to N_2 with an online elemental analyzer (PDZEuropa, ANCA-GLS) according to Mulvaney (1993), with a long-term standard deviation of 0.1‰

Gas fluxes from the top of the mesocosm were measured with a gas-tight PVC cap fitted with a silicone O-ring over 30 min. Soil pore gases were samples at 15 cm above and 15, 30, 45, 60 and 75 cm below the infiltrative surface of SND and GEO, and at 15 and 30 cm above and below the infiltrative surface for P&S (Supplemental Fig. S2.1). Concentrations of $^{15}\text{N}_2$, $^{15}\text{N}_2\text{O}$, N_2 and N_2O were determined by gas chromatography (PDZEuropa, TGII trace gas analyzer) followed by a dual inlet isotope ratio mass spectrometer (PDZEuropa 20-20 IRMS) to attain separation of N_2 and N_2O (Mosier and Schimel, 1993).

2.4 ^{15}N calculations

Natural abundance values, determined on samples taken prior to the start of the experiment, were subtracted from all enriched values. The mass of ^{15}N in gas samples was calculated according to Eq. 1:

$$[1] \quad M = \frac{At.\%}{100} \times C_g \times V_g$$

where M = mass of ^{15}N , C_g = conc. N (mg L^{-1}) in gas sample and V_g = vol. of gas sample (L).

The flux of ^{15}N per mesocosms was calculated according to Eq. 2:

$$[2] \quad \Phi = \frac{M_t - M_0}{t} \times \frac{V_h}{V_g}$$

where Φ = flux of ^{15}N (mg h^{-1}), M_t = sample mass (mg) of ^{15}N at time, t (h), M_0 = initial ^{15}N sample mass (mg), V_h = vol. of headspace (L), and V_g = vol. of gas sample (L).

The mass of ^{15}N in water samples was calculated according to Eq. 3:

$$[3] \quad \text{Mass } ^{15}\text{N} = \frac{At.\%}{100} \times C_w \times V_w$$

where M = mass of ^{15}N , C_w = conc. N (mg L^{-1}) in output water and V_w = vol. of water (L).

2.5 Travel velocity of water

Sodium chloride was added to wastewater (final conc. of 5000 mg L^{-1}) to serve as a conservative tracer, and the electrical conductivity (EC) of output water monitored continuously with an EC probe (model 06-662-61, Control Company, Friendswood, TX) inserted in a flow-through cell located at the outlet of the mesocosm (Supplemental Fig. S2.1). Breakthrough curves were used to model solute transport and determine the pore water velocity through the soil profile (Supplemental Methods).

3. RESULTS AND DISCUSSION

3.1 ^{15}N mass.

We accounted for 174%, 78%, and 97% of the ^{15}N added to the STA of P&S, SND and GEO (Table 2.3) in outputs measured over the 11, 4, and 5 days required for most of the NaCl tracer to exit the mesocosms, respectively. The mass of ^{15}N recovered in P&S was higher than the input likely due to the difficulty in measuring high N_2 against a high background, which results in overestimation of the N_2 concentration, C_g (Eq. 2) (An and Joye, 1997). Because estimates of the mass of $^{15}\text{N}_2$ were based on integration over a longer period in P&S (11 d) than in SND (4 d) and GEO (5 d), the error in the concentration of N_2 is magnified in the former. The $^{15}\text{N}_2$ concentrations were likely overestimated by a factor of two, whereas measured differences in concentration from other gases were on the order of 100 -1000 fold, therefore, the estimated values remain important to this discussion. ^{15}N was still exiting the SND and GEO mesocosms after data collection ceased, therefore we were unable to capture the complete mass introduced (Fig. 2.1).

Nitrate was the predominant aqueous species of ^{15}N recovered in all three STAs. It accounted for 48, 55 and 41% of the ^{15}N recovered in outputs from SND, GEO and P&S respectively (Table 2.3). ^{15}N -Ammonium accounted for 5.5, 6.3 and 1.5% of the ^{15}N recovered in outputs from SND, GEO and P&S, respectively. ^{15}N -Ammonium experienced little retardation relative to the NaCl tracer (Fig. 2.1), likely because of the highly acidic conditions (pH 3.5-3.8) prevalent in the soil profile (Cooper et al., 2015), which would prevent retention by cation exchange. ^{15}N -Nitrate

in drainage water was detected after $^{15}\text{NH}_4^+$, reflecting the time it takes for microbial oxidation (Fig. 2.1).

The mass of $^{15}\text{N}_2$ dissolved in drainage water was greater than that of $^{15}\text{N}_2\text{O}$ for all STAs (Table 2.3). We had previously reported 20-30 $\mu\text{g N L}^{-1}$ of dissolved N_2O in drainage water (Cooper et al., 2015), which is in good agreement with the 11-29 $\mu\text{g }^{15}\text{N L}^{-1}$ dissolved N_2O in drainage water recorded in this study. The concentration of dissolved N_2 in drainage water was 1,000 times higher than that of N_2O , ranging from 9-11 mg N L^{-1} in SND and GEO, and 17-29 mg N L^{-1} in P&S. The presence of dissolved $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ in drainage water indicates *in situ* production of these gases – rather than just transport of gases initially dissolved in wastewater inputs – as hypothesized in Cooper et al. (2015).

Gas losses at the soil-atmosphere interface were dominated by N_2 in all three drainfield types (Table 2.3). However, the amounts of $^{15}\text{N}_2$ lost in the gas and dissolved phases differed between STA types. Losses of $^{15}\text{N}_2$ were higher in gas than dissolved phases in P&S, whereas losses in the dissolved phase were greater than the gas phase in SND and GEO. This may be due to the higher average velocity of the wastewater in SND and GEO than P&S (Supplemental Fig. S2.2), which would diminish diffusion out of the top of the mesocosm, and to the production of $^{15}\text{N}_2$ at all depths of the soil profile in SND and GEO (Fig. 2.2). In contrast, a higher mass of $^{15}\text{N}_2\text{O}$ was lost in the gas phase relative to dissolved losses in all STAs, likely because most N_2O production was near the surface, as discussed below.

3.2 $^{15}\text{N}_2$ gas flux

Positive flux of $^{15}\text{N}_2$ was detected at the first sampling time after introduction of $^{15}\text{NH}_4^+$ to all drainfield types: 1 h for SND and GEO and 4 h for P&S (Fig. 2.1).

We determined in a preliminary experiment that the travel time for sulfur hexafluoride (SF_6), a conservative gas tracer, to be 1 h for SND and GEO and 4 h for P&S,

comparable to the time between $^{15}\text{NH}_4^+$ introduction and measureable $^{15}\text{N}_2$ fluxes.

Based on modeled estimates of the velocity of water in the mesocosms (Supplemental Figure S2.2, S2.3), N_2 gas was produced within the top 1 cm in all STA types.

In the P&S drainfield $^{15}\text{N}_2$ flux peaked periodically, with the time between peaks and the magnitude of each peak increasing over time. Heterotrophic denitrification is likely to be a substantial contributor to the $^{15}\text{N}_2$ flux in P&S because of high C availability (Table 2.2) in the upper portion of the soil profile. Autotrophic denitrification is possible throughout the soil profile because CO_2 is present in soil pore gas throughout the STA. Some autotrophic denitrifiers (e.g. sulfur-utilizing) have been found to be competitive with heterotrophic denitrifiers (Oh et al., 2001).

Although heterotrophic denitrification out-competes anammox when organic C is available (Chamchoi et al., 2008), anammox is a potential contributor to N loss at greater depths in the STA and may contribute to peaks in flux later in the experimental timeline. The periodic cycling of $^{15}\text{N}_2$ flux may be related to changes in soil conditions and the availability of C and N substrates as water moves through the soil profile, which may be linked to internal recycling of C and N through microbial biomass. Additional data would be necessary to pinpoint the processes involved.

Peaks in $^{15}\text{N}_2$ flux in SND were observed 6 and 96 h after introduction of $^{15}\text{NH}_4^+$. The first peak of $^{15}\text{N}_2$ flux corresponds to passage of the ^{15}N tracer through the top 16 cm of soil in SND (Supplemental Fig. S2.3), however, the second peak occurred after the ^{15}N tracer began exiting the mesocosm (Fig. 2.1). In contrast, the flux of $^{15}\text{N}_2$ from GEO had four distinct peaks, at 3, 24, 72 and 120 h after addition of $^{15}\text{NH}_4^+$. The first two peaks in GEO correspond to soil profile depths (7 and 51 cm, Fig. 2.2), however, the last two peaks occurred after ^{15}N tracer began to exit the mesocosm.

Both SND and GEO receive SFE, which has a low concentration of organic C (Table 2.2) that likely limits heterotrophic denitrification to the soil below the infiltrative area, corresponding to the first peak. Subsequent peaks are from processes deeper in the soil profile, where organic C is likely to be depleted and autotrophic processes don't have to compete with heterotrophic processes. Areas of increased moisture deeper in the soil profile may result in anoxic conditions that support autotrophic denitrification, anammox, or feammox. The soils in our mesocosms have a marked textural discontinuity between the B (silt loam) and C horizons (very gravelly coarse sand) (Supplemental Fig. S2.4) where soil water content is expected to fluctuate periodically. As water moves down the soil profile, capillary suction causes it to collect in the finer textured soil above the boundary. The results in a temporary increase in moisture content, limiting O_2 diffusion, and establishing hypoxic conditions. Once the soil water content is sufficiently high above the textural discontinuity, the force of gravity overcomes capillary suction, and the water drains

into the horizon below. The textural discontinuity is not a factor in P&S, where deeper placement of the infiltrative surface was exclusively in the C horizon.

3.3 $^{15}\text{N}_2\text{O}$ gas flux

Positive flux of $^{15}\text{N}_2\text{O}$ occurred within 48-72 h of $^{15}\text{NH}_4^+$ introduction to the infiltrative area for all STA types (Fig. 2.1). Two peaks in $^{15}\text{N}_2\text{O}$ flux were observed in P&S: a smaller one produced within 4 h of introduction of $^{15}\text{NH}_4$, and a larger one after the next STE dose was complete, 12 h after $^{15}\text{NH}_4^+$ introduction. Both peaks occurred within the top 7 cm of the STA (Supplemental Fig. S2.3). Furthermore, the time course was the same for $^{15}\text{N}_2\text{O}$ and $^{15}\text{N}_2$ flux, indicating that a process that produces both gases, such as heterotrophic denitrification, was at work.

A single peak of $^{15}\text{N}_2\text{O}$ flux was observed in SND and GEO, at 24 and 1 h respectively, with $^{15}\text{N}_2\text{O}$ flux from SND, over an order of magnitude higher than GEO. The SND peak corresponds to a depth of 50 cm, while the peak flux of $^{15}\text{N}_2\text{O}$ in GEO corresponded to a depth of 1 cm below the infiltrative surface (Fig. 2.2).

The dosing mechanism differs between SND and GEO, with GEO designed to allow greater O_2 diffusion by slow wastewater infiltration through a diffuser and filter fabric. Higher availability of O_2 in the GEO infiltrative area may result in quick consumption of organic C, making C less available. This would limit heterotrophic denitrification, and thus production of $^{15}\text{N}_2\text{O}$. Further along the experimental time course in all STAs, no substantial production of $^{15}\text{N}_2\text{O}$ was observed, but $^{15}\text{N}_2$ continued to be produced (Fig. 2.1), suggesting that processes producing little to no N_2O (e.g. autotrophic S-linked denitrification, anammox, feammox) are at work.

3.4 Contributions of $^{15}\text{N}_2\text{O}$ to nitrification and denitrification

Both nitrification and denitrification produce N_2O . Autotrophic denitrification coupled to sulfur oxidation produces N_2O to a lesser extent than the heterotrophic process (Park et al., 2002), whereas neither anammox nor feammox produce N_2O (Van de Graaf et al., 1995; Ding, et al., 2014). For both nitrification and denitrification, production of N_2O increases with decreasing O_2 concentration (Wrage, 2001; Wunderlin et al. 2012).

The ratio of N_2O to N_2 produced from heterotrophic denitrification under anoxic conditions is reported to be between 0.11 and 0.12 (Khalil et al. 2002; Richard, et al. 2014), with N_2O production over this ratio attributed to nitrification. The $^{15}\text{N}_2\text{O}:^{15}\text{N}_2$ ratio in our experiment was between 9.5×10^{-6} and 1.0×10^{-3} for all STA types, two to five orders of magnitude below the ratio for denitrification under anoxic conditions. This very low ratio suggests that heterotrophic denitrification is unlikely to be the only process at work producing N_2 in the STA.

3.5 ^{15}N soil pore gas profiles

If $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ are only produced at the infiltrative surface we would expect a decline in their concentration with depth, resulting from diffusion and/or consumption. We observed that the concentration of these gases either remained constant or increased with depth, an indication that *in situ* production of $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ took place within the soil profile (Fig. 2.2). Furthermore, the spatial distribution of $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ production within the soil profile appear to have

opposing drivers (Fig. 2.2), as suggested by peaks in concentration at different depths for N_2 and N_2O .

Production of N_2 from unenriched (^{14}N) and enriched (^{15}N) pools produce gases with different isotope ratios, or isotopologues: m/z 28 (^{14}N - ^{14}N), m/z 29 (^{14}N - ^{15}N), and m/z 30 (^{15}N - ^{15}N). If only one process produced N_2 in each STA type, the ratio of enriched (29, 30) to unenriched (28) gas is expected to increase consistently with depth; however, this was not the case (Fig. 2.3). Denitrification, both heterotrophic and autotrophic, form N_2 from two molecules of NO_3^- , which must be adjacent to sites of denitrification. In a poorly mixed system, like soil, a concentrated aliquot of $^{15}NH_4^+$ is likely to produce $^{15}NO_3^-$ molecules in close proximity, with N_2 production from denitrification likely to form more m/z 30 than 29. Conversely, anammox produces N_2 from NO_2^- and NH_4^+ , with addition of $^{15}NH_4^+$ more likely to form more m/z 29 than 30. The $^{14}NO_2^-$ present in solution is more likely to react with $^{15}NH_4^+$ because $^{15}NO_2^-$ will be present in a smaller quantity than $^{14}NO_2^-$. A plot of 30/28 (Fig. 2.3) shows more ^{15}N - ^{15}N production 15 cm below the infiltrative surface of P&S, likely from heterotrophic denitrification. Higher levels of 30/28 in SND and GEO were produced at the upper and lower portions of the soil profile, potentially from heterotrophic and autotrophic denitrification, respectively. A plot of 29/28 (Fig. 2.3) shows production of ^{15}N - ^{14}N increases with depth in all STA types, potentially from increased anammox activity lower in the soil profile where competition from heterotrophic denitrification is minimal.

We examined the relationship between potential controlling factors and the concentration of $^{15}N_2$ and $^{15}N_2O$ in the soil profile (Table 2.4). Production of $^{15}N_2$ was

positively and significantly correlated with O_2 , likely because O_2 availability limits nitrification, and thus denitrification. Methane concentration also appeared to be an important factor controlling $^{15}N_2$, with the significant negative correlation between methane concentration and N_2 concentration likely due to competitive inhibition of nitrification by CH_4 (Bedard and Knowles, 1989). This would restrict N_2 formation by denitrification, and N_2O production from nitrification or denitrification. The concentration of CO_2 was also negatively correlated with $^{15}N_2$ production. High CO_2 production is expected in the STA from microbial decomposition and respiration, therefore, a significant negative correlation may be due to consumption of CO_2 by autotrophs, including those that produce N_2 . Production of $^{15}N_2O$ within the STA was not significantly correlated with any of the factors we measured (Table 2.4). This was likely due to the complex nature of N_2O production, with potential contributions from aerobic (nitrification) and anaerobic (N reducing) processes, making it unlikely that a single factor controls its production.

Surprisingly, no significant correlations were found between the concentration of N_2 or N_2O and water filled pore space (WFPS) (Table 2.4, Fig. 2.2), which is often cited as an important constraint for N removal by denitrification (Motz et al., 2012; Bateman and Baggs, 2005). This raises questions about the impact of WFPS on N dynamics, and suggests that establishment of anoxic conditions involves additional factors, such as low DO levels of wastewater inputs and/or rapid consumption of oxygen by aerobic microbial processes relative to diffusion.

3.6 Inorganic N dynamics

If ratios of $^{15}\text{NH}_4^+$ or $^{15}\text{NO}_3^-$ relative to the NaCl concentration over time are considered, points of production and consumption of N species can be identified, which can provide insights into possible removal processes (Fig. 2.4). The ratio of $^{15}\text{NH}_4^+/\text{NaCl}$ is generally expected to decrease as a result of consumption, whereas $^{15}\text{NO}_3^-/\text{NaCl}$ is expected to increase due to production by nitrification. In Figure 2.4, letters represent samples taken at progressive time points (A-D).

Between time points B and C, $^{15}\text{NH}_4^+$ concentrations decrease in all STAs, while $^{15}\text{NO}_3^-$ concentrations did not increase in any STA type. This was unexpected because the assumption was that NH_4^+ is quickly nitrified. Since this was not observed, processes other than nitrification must be consuming ammonium, such as microbial uptake and anammox/feammox. We observed an increase in $^{15}\text{NH}_4^+$ between time points C and D, 96 h (P&S) and 24 h (SND and GEO) after consumption was observed. Re-mineralization of microbial biomass N, NH_4^+ in excretions from predators, and dissimilatory nitrate reduction could cause this increase in $^{15}\text{NH}_4^+$ concentration (Fig. 2.4), suggesting a rapid turnover of the soil microbial community. This has consequences for the determination of N removal, and highlights the importance of measuring N removal rates at time scales longer than turnover times to assess removal accurately. Measurements made at intervals shorter than turnover times may incorrectly identify temporary uptake as permanent removal.

4. CONCLUSIONS

1. We found both conventional (P&S) and advanced (SND and GEO) soil treatment areas produced $100 - 1000 \times$ more $^{15}\text{N}_2$ than $^{15}\text{N}_2\text{O}$, despite the differences in composition of wastewater inputs, placement of the STA within the soil profile, and soil physicochemical conditions.
2. We observed N_2 and N_2O production both at the infiltrative surface and at greater depths in all three STA types, as indicated by the timing of peaks in ^{15}N gas flux and the vertical distribution of these gases in the soil profile.
3. Our results suggest that both autotrophic and heterotrophic processes contribute to N losses in all STA types.
4. It appears that there is internal recycling of ammonium and nitrate within all STA types.
5. Our results show that N transformations within the STA are complex, with multiple processes likely contributing to N removal. Elucidation of the specific mechanisms, their relative contribution, and their interactions is necessary to gain a clear understanding of N removal processes and improve their efficiency.

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TABLES

Table 2.1 Minimal processes that can contribute to production of N₂ and N₂O in the soil treatment area of onsite wastewater treatment systems (OWTS).

Process	C source	Electron donor	Electron acceptor	Relative production of N ₂ and N ₂ O
Heterotrophic denitrification	Organic	Organic	NO ₃ ⁻	N ₂ >N ₂ O
CH ₄ -coupled denitrification	CO ₂	CH ₄	NO ₃ ⁻	N/A *
S-coupled denitrification	CO ₂	H ₂ S, S ⁰	NO ₃ ⁻	N ₂ >>N ₂ O
Anaerobic ammonia oxidation	CO ₂	NH ₄ ⁺	NO ₂ ⁻	N ₂ only
Iron-coupled ammonia oxidation	CO ₂	NH ₄ ⁺	Fe ³⁺	N ₂ only

*Not available

Table 2.2 Characteristics of septic tank effluent (STE) for the pipe and stone (P&S) soil treatment area, and sand filter effluent (SFE) for shallow narrow (SND) and Geomat ® (GEO) soil treatment areas used in this study. Units are mg L⁻¹ except for electrical conductivity (µS/cm) and pH.

Property	P&S		SND		GEO	
	Input	Output	Input	Output	Input	Output
pH	6.9	3.7	5.6	3.4	5.4	3.5
Dissolved oxygen	0.1	0.0	3.1	2.0	3.4	0.6
5-day biochemical oxygen demand	190	0.0	4.0	0.0	12	0.0
Total suspended solids	46	0.0	2.0	0.0	2.0	0.0
Electrical conductivity	880	750	470	560	470	550
Total N	74	70	54	47	61	41
NH ₄ ⁺ -N	53	0.10	15	2.5	14	3.1
NO ₃ ⁻ -N	0.05	57	10	32	27	38
SO ₄ ²⁻ -S	7.2	8.4	29	9.4	21	8.8

Table 2.3 Mass and of ^{15}N recovered in aqueous and gas pools from pipe and stone (P&S), shallow narrow (SND) and Geomat ® (GEO) soil treatment areas over 11, 4 and 5 days respectively. Inputs of ^{15}N were 420 μg for P&S, 482 μg for SND and 391 μg for GEO.

Nitrogen Species	P&S		SND		GEO	
	Mass* (μg)	Fraction of ^{15}N recovered (%)	Mass (μg)	Fraction of ^{15}N recovered (%)	Mass (μg)	Fraction of ^{15}N recovered (%)
NO_3	296	40.5	183	54.6	183	48.3
NH_4	11	1.5	21	6.3	21	5.5
$\text{N}_2\text{-N}$ flux	338	46.4	37	11.0	13	3.4
$\text{N}_2\text{O-N}$ flux	0.11	<1.0	0.15	<1.0	0.009	<1.0
Diss. $\text{N}_2\text{-N}$	85	11.6	94	28.1	162	42.7
Diss. $\text{N}_2\text{O-N}$	0.025	<1.0	0.065	<1.0	0.072	<1.0
Sum of N species	730		335		379	

* Total ^{15}N mass in output water was estimated by integration under the curve of ^{15}N concentration ($\mu\text{g L}^{-1}$) vs. time (h), and multiplying by discharge, Q (L h^{-1}). Total ^{15}N mass in output gas was estimated by integration under the curve of ^{15}N flux ($\mu\text{g h}^{-1}$) vs. time (h).

Table 2.4 Spearman correlation coefficient (R) of $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ with O_2 , CH_4 , CO_2 , and water filled pore space (WFPS) within the soil profiles of pipe and stone (P&S), shallow narrow (SND) and Geomat ® (GEO) soil treatment areas. Values are for samples take 48 h after dosing with $^{15}\text{NH}_4^+$. Values in bold indicate significant correlation ($p < 0.10$).

Analyte	$^{15}\text{N}_2$		$^{15}\text{N}_2\text{O}$	
	R	P value	R	P value
O_2	0.584	0.067	-0.334	0.327
CH_4	-0.673	0.029	-0.418	0.213
CO_2	-0.782	0.005	0.248	0.468
WFPS	0.214	0.578	-0.524	0.160

FIGURES

Fig. 2.1 Time-course of NaCl tracer, ^{15}N -ammonium, ^{15}N -nitrate, $^{15}\text{N}_2$ flux and $^{15}\text{N}_2\text{O}$ flux for pipe and stone (P&S), shallow narrow (SND) and Geomat ® (GEO) soil treatment areas.

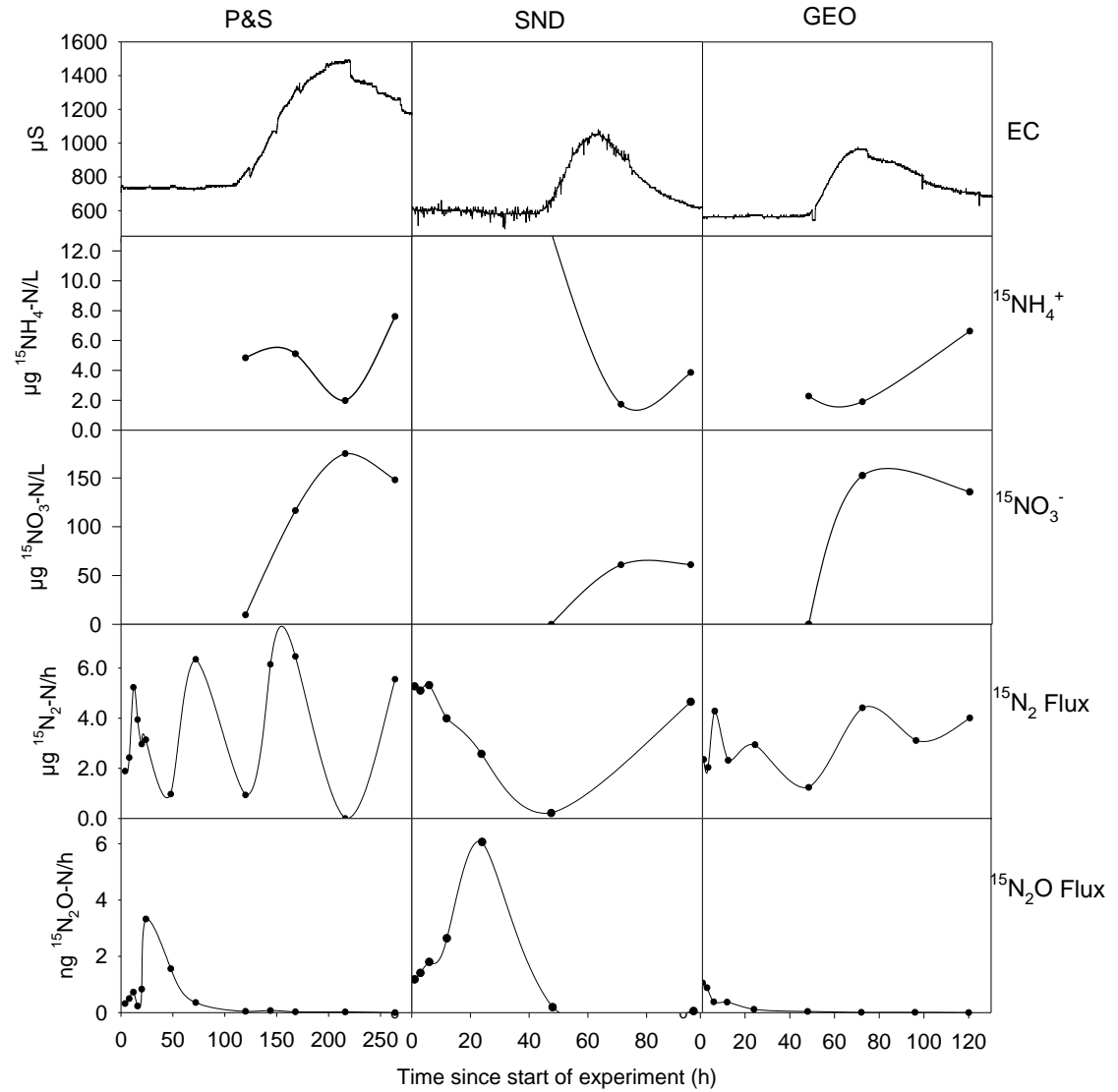


Fig. 2.2 Profiles of concentration of $^{15}\text{N}_2$, $^{15}\text{N}_2\text{O}$, O_2 , CH_4 and CO_2 in soil pores and water filled pore space (WFPS) for pipe and stone (P&S), shallow narrow (SND) and Geomat[®] (GEO) soil treatment areas at 120 h (P&S) and 48 h (SND and GEO) after addition of $^{15}\text{NH}_4^+$. Note the unconnected point at 0 cm is in the infiltrative headspace rather than within the soil profile. Depth is measured from the infiltrative surface.

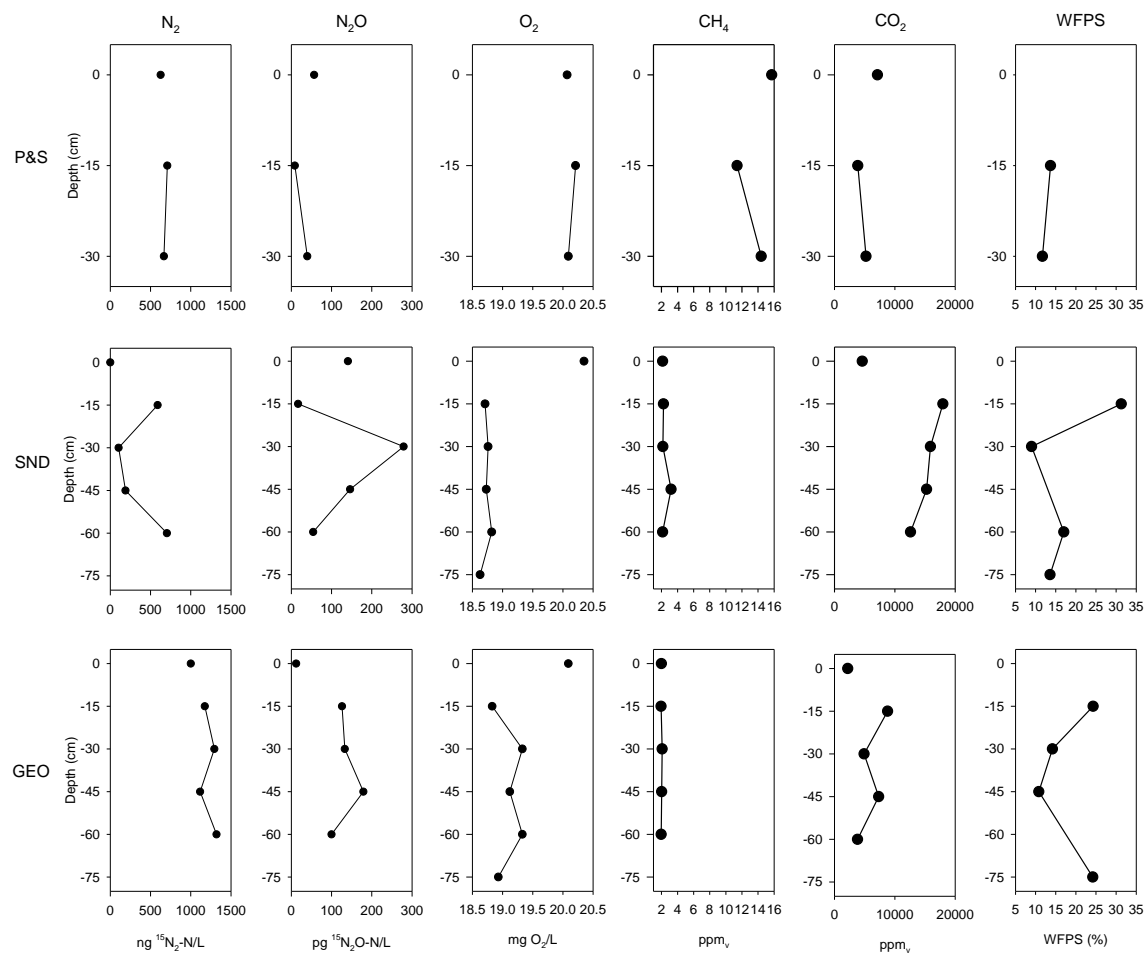


Fig. 2.3 Ratios of $^{30}\text{N}_2$ (^{15}N - ^{15}N) and $^{29}\text{N}_2$ (^{15}N - ^{14}N), to unenriched $^{28}\text{N}_2$ (^{14}N - ^{14}N) for pipe and stone (P&S), shallow narrow (SND) and Geomat ® (GEO) soil treatment areas. The top unconnected point is in the infiltrative headspace rather than the soil profile. Depth is measured from the infiltrative surface.

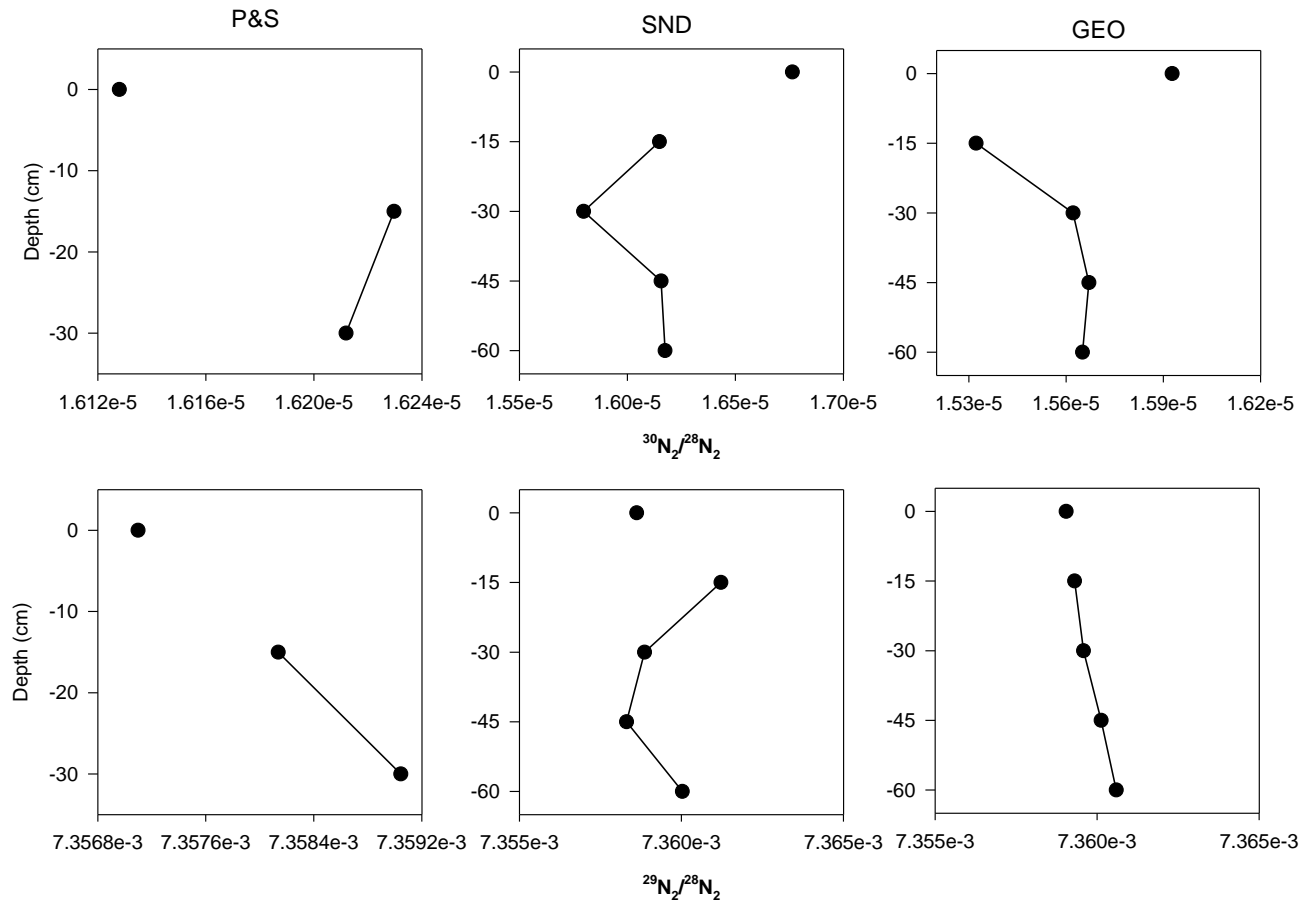
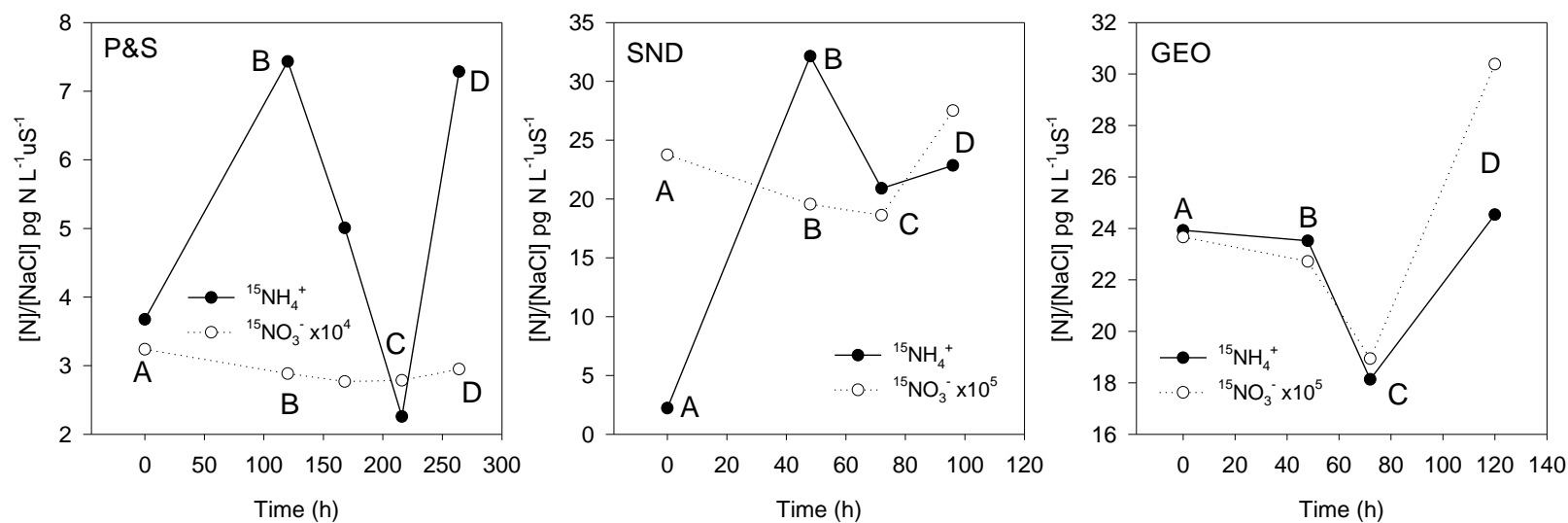
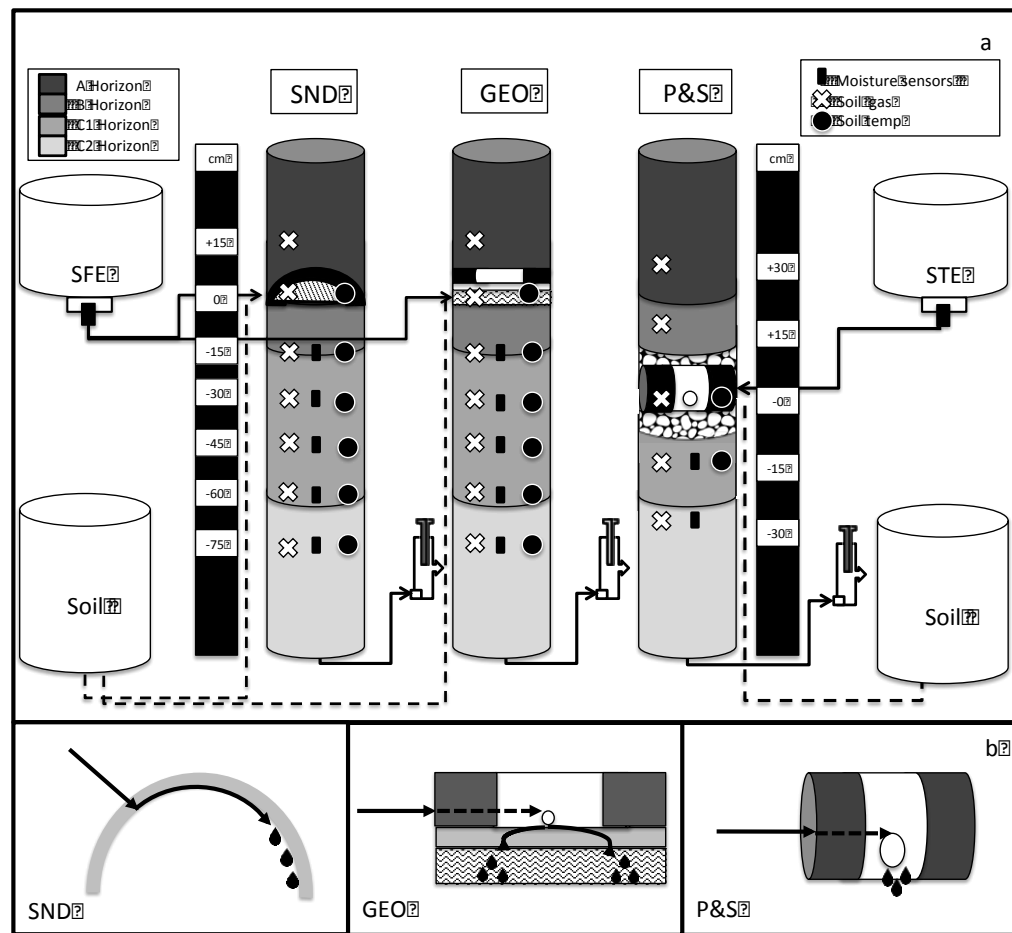


Fig. 2.4 Changes in the ratio of $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ to NaCl over time. Letters represent samples taken at progressive time points (A-D) for pipe and stone (P&S), shallow narrow (SND) and Geomat[®] (GEO) soil treatment areas.

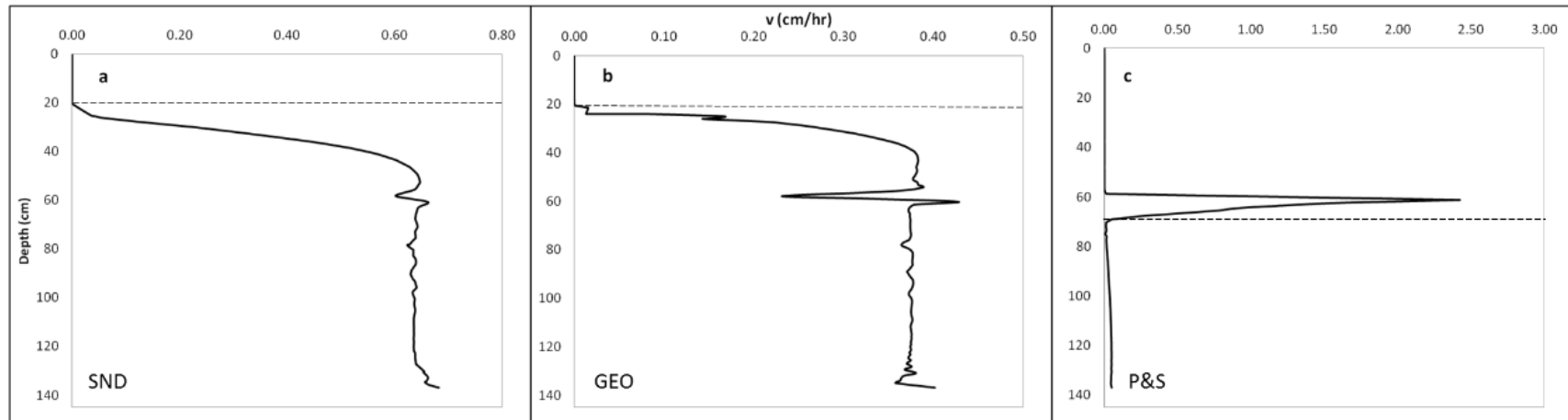


SUPPLEMENTAL MATERIAL

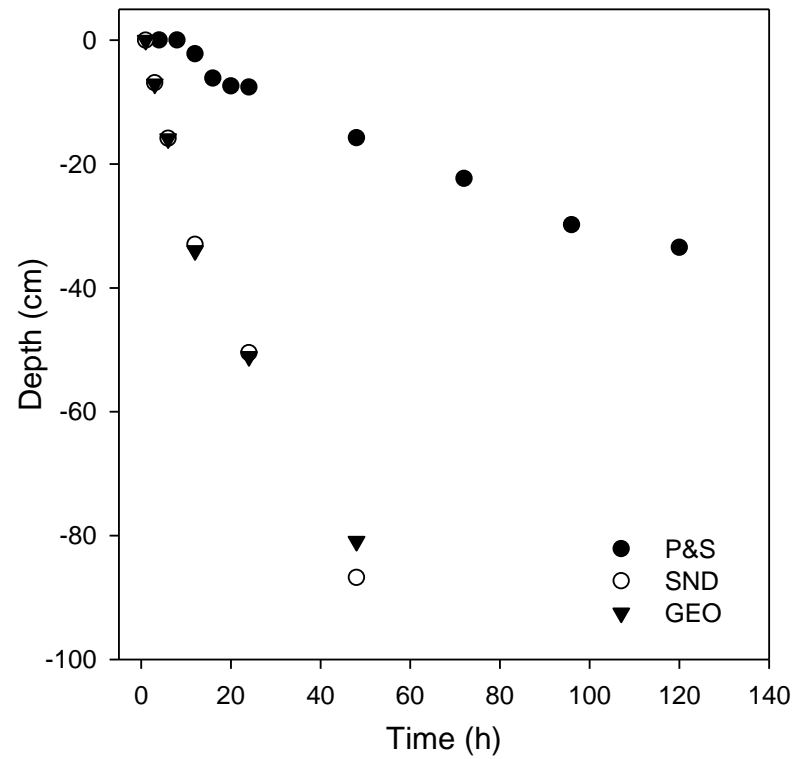
Supplemental Fig. S2.1. (a) Schematic diagram of soil mesocosms representing a shallow narrow (SND), GeoMat® (GEO), and pipe and stone (P&S) soil treatment areas. The wastewater input to SND and GEO was sand filter effluent (SFE), whereas the P&S received septic tank effluent (STE). The approximate location of soil horizons, ports for gas sampling, and moisture and temperature probes are indicated. Water exits the mesocosms through a hanging water column device used to adjust the height of the water table. The atmosphere in the infiltrative area is connected to a 30-cm soil column. (b) Detailed schematic diagram of the SND, GEO and P&S delivery devices. Diagrams are not to scale.



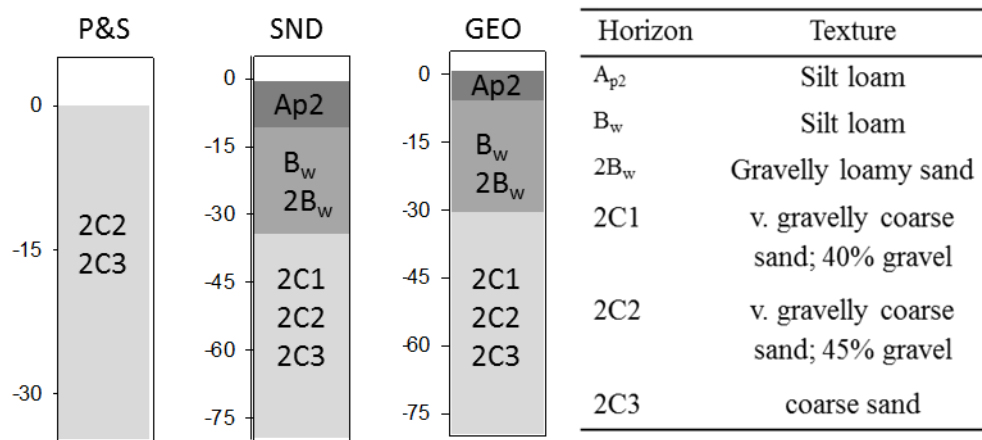
Supplemental Fig. S2.2 Pore-water velocity within the soil treatment area of (a) shallow narrow (SND), (b) Geomat ® (GEO) and (c) pipe and stone (P&S). Calculated average velocities (cm h^{-1}) are 0.64, 0.37 and 0.03, for SND, GEO and P&S, respectively. Dashed line represents the infiltrative surface. Depth is measured from the soil surface.



Supplemental Fig. S2.3 Depth of NaCl tracer as a function of time for pipe and stone (P&S), shallow narrow, and Geomat[®] (GEO) soil treatment areas. Depth is measured from the infiltrative surface.



Supplemental Fig. S2.4 Depth profile of soil textural classes for pipe and stone (P&S), shallow narrow (SND) and Geomat ® (GEO) soil treatment areas. Depth is measured from the infiltrative surface.



Supplemental Methods

HYDRUS 2D/3D version 2.0 was used to simulate water flow and NaCl transport through the soils under variably-saturated conditions. HYDRUS is a commercially-available computer program used to simulate water flow, solute and microbial transport, heat transport, and colloid transport in variably-saturated porous media. The program numerically solves the Richards equation for saturated-unsaturated water flow (Eq. 1):

$$\frac{\partial \theta}{\partial t} = \frac{\partial}{\partial x_i} \left[K \left(K_{ij}^A \frac{\partial h}{\partial x_j} + K_{iz}^A \right) \right] - S \quad (1)$$

where θ is the volumetric water content [L^3L^{-3}], h is the pressure head [L], S is a sink term [T^{-1}], x_i ($i=1,2$) are the spatial coordinates [L], t is time [T], K_{ij}^A are components of a dimensionless anisotropy tensor K^A , and K is the unsaturated hydraulic conductivity function [LT^{-1}] given by

$$K(h, x, y, z) = K_s(x, y, z) K_r(h, x, y, z) \quad (2)$$

where K_r is the relative hydraulic conductivity and K_s the saturated hydraulic conductivity [LT^{-1}]. The model permits the application of the convection - dispersion equation in the liquid phase to simulate solute transport and fate. Chemical equilibrium and linear adsorption is described by the following mass balance equation:

$$\frac{\partial \theta c}{\partial t} + \rho K_d \frac{\partial c}{\partial t} = \frac{\partial}{\partial x} \left(\theta D_{ij}^w \frac{\partial c}{\partial x_j} \right) + \frac{\partial}{\partial z} \left(\theta D_{ij}^w \frac{\partial c}{\partial z_j} \right) - \frac{\partial q_x c}{\partial x} - \frac{\partial q_z c}{\partial z} - \mu \theta c \quad (3)$$

where c is dissolved solution concentration [ML^{-3}], t is time (T), K_d is the adsorption coefficient (L^3M^{-1}), μ represents the solute transformation or degradation rate in the

liquid phase, x is the solute travel distance (L) and z is depth (L). D_{ij}^w is the dispersion coefficient tensor for the liquid phase [L^2T^{-1}], θ is the volumetric water content [L^3L^{-3}], ρ is the bulk density of porous medium [ML^{-3}], and q_x and q_z is the specific discharge [LT^{-1}] along the horizontal and vertical direction, respectively.

**MANUSCRIPT – III: HELL AND HIGH WATER: DIMINISHED SEPTIC
SYSTEM PERFORMANCE DUE TO CLIMATE CHANGE**

In preparation for submission to PLOS ONE

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ABSTRACT

Climate change may reduce the ability of soil-based onsite wastewater treatment systems (OWTS) to treat wastewater. Higher temperatures and water tables can affect treatment by reducing the volume of unsaturated soil and oxygen available for treatment, which may result in greater transport of pathogens, nutrients, and biochemical oxygen demand (BOD₅) to groundwater, jeopardizing public and aquatic ecosystem health. The soil treatment area (STA) of an OWTS removes contaminants as wastewater percolates through the soil profile. Conventional STAs receive wastewater from the septic tank, with infiltration occurring deeper in the soil profile. In contrast, shallow narrow STAs receive pre-treated wastewater that infiltrates higher in the soil profile, which may make them more resilient to climate change. We used intact soil mesocosms to quantify the impact of climate change (30 cm increase in water table, 5°C increase in soil temperature) on the water quality functions of a conventional STA and two types of shallow narrow STAs. Greater removal of BOD₅ was observed under climate change for all STA types. No fecal coliform bacteria (FCB) were released under current climate, whereas as many as 17 and 20 CFU 100 mL⁻¹ were released in conventional and shallow narrow drainfields, respectively, under climate change. Phosphorus removal decreased from 75% under present climate to 66% under climate change in the conventional STA, and from 100% to 71-72% shallow narrow STAs. Total N removal increased from 14% under present climate to 19% under climate change in the conventional STA, but decreased in shallow narrow STAs, from 6-7% under present climate to less than 3.0%. Leaching of N in excess of inputs was also observed in shallow narrow STAs under climate change. Our results

indicate that climate change can affect contaminant removal, with effects dependent on the contaminant and STA type.

INTRODUCTION

The soil treatment area (STA; also known as a drainfield or leachfield) of an onsite wastewater treatment system (OWTS) is an important component for removal of contaminants from wastewater. Treatment takes place as wastewater percolates through the unsaturated portion of the soil profile, where low moisture and high oxygen (O_2) levels are conducive to removal of pathogens and where chemical and microbial processes can reduce the concentration of other contaminants. The extent of treatment in the STA depends on the volume of unsaturated soil the wastewater passes through, represented by the vertical separation between the infiltrative surface of the STA and the water table [1-4]. Because wastewater renovation relies on hydrologic, microbial and chemical processes, treatment of wastewater in the STA is sensitive to changes in soil moisture and temperature.

Wastewater contains contaminants that affect human and environmental health. Pathogenic microorganisms (bacteria, viruses, protozoa and nematodes) can cause illness in humans from ingestion or contact with contaminated water. Excessive nitrate concentration in drinking water disrupts O_2 binding to red blood cells, known as methemoglobinemia [5], and may cause breathing difficulties in infants. Inputs of nitrogen (N) and phosphorus (P) from OWTS to aquatic ecosystems contribute to eutrophication [6] in marine and fresh waters, respectively. Release of biodegradable organic carbon, as biochemical oxygen demand (BOD_5), promotes microbial consumption of available O_2 , resulting in hypoxia and death of aquatic organisms [7].

Climate change, through the combined effects of temperature and sea level rise, is expected to be detrimental to contaminant removal in the STA. Sea level rise will reduce the volume of unsaturated soil available for wastewater treatment in coastal areas. The sea level in the Northeastern U.S. is projected to rise 90-120 cm by 2100 [8], resulting in higher water tables in coastal regions when denser saltwater displaces lighter freshwater. Furthermore, precipitation events are expected to increase in number and severity over the same time period [8]. Higher groundwater tables and increased precipitation will result in a wetter conditions that enhance the survival and transport of bacterial and viral pathogens [9,10]. Wetter soils may also result in metal reduction, leading to lower P removal capacity and increased mobilization of P retained on soil particles [11]. In contrast, removal of N by microbial reduction to N_2 may be enhanced by diminished O_2 diffusion in wetter soils. Finally, decomposition of organic carbon may be hindered or enhanced by increased soil moisture [12], which will affect BOD_5 removal. Because 40% of the U.S. population resides in coastal communities [13], sea level rise will likely impact coastal communities that rely on OWTS for wastewater renovation.

Elevated temperatures due to climate change may also affect contaminant removal in the STA. Atmospheric temperature is expected to increase 3-5 °C in the next 100 years in the Northeastern U.S, warming the soil profile [8], and warmer conditions have been shown to increase bacterial and viral pathogen mortality [14,15]. Warmer temperature will increase microbial activity, which may enhance removal of BOD_5 , but lower levels of BOD_5 may limit heterotrophic processes such as N removal by denitrification. Higher temperatures will also reduce O_2 solubility and increase

microbial O₂ consumption, resulting in less O₂ available for aerobic treatment processes. In addition, this reduction in available O₂ will likely lead to low redox conditions, resulting in metal reduction and a diminished P removal capacity of soil.

In a conventional OWTS, solids are removed from wastewater by sedimentation in the septic tank, and septic tank effluent (STE) is dispersed to the STA for final treatment. The STA in a conventional OWTS is located deep in the soil profile – generally in the C horizon – where infiltration of STE into coarser textured soil with larger pores reduces the likelihood of hydraulic failure due to clogging. Shallow narrow drainfields, an alternative type of STA used with advanced treatment technologies, may be more resilient to climate change effects than conventional STAs. A shallow narrow STA receives effluent that has undergone secondary treatment in an advanced treatment component, resulting in higher dissolved oxygen levels, and reduced levels of BOD₅ and particulates prior to STA dispersal. The secondary treatment lowers the probability of hydraulic failure due to clogging of soil pore spaces and allows the infiltrative surface to be placed higher in the soil profile than in a conventional STA. Shallower dosing affords a greater volume of unsaturated soil for treatment, and may provide better oxygenation, as well as enhanced filtration of wastewater through finer soil particles in the upper portion of the soil profile. In addition, shallow narrow STA designs incorporate frequent timed-dosing of small volumes of wastewater, preventing prolonged periods of soil saturation, which are common in a conventional STA. Together, these factors may make shallow narrow STAs more resilient to climate change than conventional STAs.

In a previous study we compared the water quality functions of conventional and shallow narrow STAs [16]. We observed complete removal of fecal coliform bacteria (FCB), bacteriophage (a human virus surrogate) and total P, and near complete removal of BOD₅ in conventional and shallow narrow STAs [16]. Although limited, removal of total N was higher in the conventional STA [16]. In the present study we tested the hypothesis that climate change (higher water table and increased temperature) would diminish removal of FCB, viral surrogates, and total P in conventional and shallow narrow STAs, whereas removal of BOD₅ and total N would be marginally improved. We expected the shallow narrow STAs to have comparatively better contaminant removal than conventional STAs because the former have a larger volume of soil for treatment. We evaluated these hypotheses in a laboratory experiment using triplicate intact soil mesocosms representing a conventional STA and two types of shallow narrow STAs. We compared the water quality functions of the STAs under present climate (20°C; vertical separation distance representative of regulatory values), and climate change (25°C; vertical separation distance reduced by 30 cm by raising the water table elevation) conditions. These results should be representative of the expected response of OWTS to climate change in the glaciated Northeastern U.S.

MATERIALS AND METHODS

Description of mesocosms

We used intact soil mesocosms (152-cm tall × 15-cm-dia.) to represent three STA types: (i) conventional pipe and stone (P&S), (ii) shallow narrow drainfield (SND), and (iii) Geomat® (GEO), a SND variation (S3.1 Fig). The infiltrative area

was established at 20 cm below the ground surface for SND (S3.1 Fig), at 25 cm for GEO, and at 84 cm for P&S. A detailed description of the experimental design, sampling, and analytical methods can be found in [16].

Moisture

The separation distance from the water table was controlled using a hanging water column (S3.1 Fig) and, to represent present climate conditions, was set at 102 cm below the infiltrative surface for SND and GEO, and at 56 cm for P&S. To simulate climate change, the water table elevation was raised 30 cm, resulting in a separation distance of 72 cm for SND and GEO, and 26 cm for P&S. Soil EC-5 moisture probes and Em5b data loggers (Decagon Devices, Pullman, WA) were used to measure soil moisture every 15 minutes at depths of 15, 30, 45, 60 and 75 cm below the infiltrative surface for SND and GEO, and at 15 and 30 cm below the infiltrative surface for P&S (S3.1 Fig).

Temperature

The mesocosms were maintained at 20.0 ± 0.7 °C under present climate conditions, and the temperature increased to 25.0 ± 0.7 °C to simulate climate change. This was accomplished by covering the outside of the mesocosms with heavy-duty aluminum foil (to increase heat diffusion), wrapping 115V heating cables (Hydrokable, Sacramento, CA) around the mesocosms, and wrapping reflective double bubble foil insulation material around the mesocosms. A thermostat (NEMA 4X, Aqua Logic, Inc., San Diego, CA) was used to regulate the temperature, and soil temperature was measured using iButton sensors (DS1921G, Maxim Integrated, San

Jose, CA) buried 5 cm below the soil surface. Ceramic-tipped probes (YSI, Yellow Springs, OH) were used to measure soil temperature at depths of 0, 15, 30, 45, 60 and 75 cm below the infiltrative surface for SND and GEO, and at 0 and 15 cm below the infiltrative surface for P&S (S3.1 Fig).

Wastewater dosing and characteristics

The P&S was dosed with 200 mL of septic tank effluent (STE) every 12 h over 1.5 h, corresponding to 400 mL d⁻¹ (22.6 L m⁻² d⁻¹). The SND and GEO received wastewater that had passed through a single-pass sand filter (SFE). They were dosed with 22.5 mL SFE every 30 min over 15 min, corresponding to 2000 mL d⁻¹ (113 L m⁻² d⁻¹). Septic tank effluent and SFE were collected weekly from the same treatment train at a residence in South Kingstown, RI, USA. The characteristics of wastewater inputs (Table 3.1) are within the range observed by others [17-19].

Analyses

Output water was collected at the bottom of the mesocosms under both climate conditions, in N₂-purged, autoclaved 1-L Nalgene bottles fitted with an airlock, and the water was analyzed for pH, dissolved O₂, BOD₅, electrical conductivity, FCB, total N, ammonium, nitrate, total P, phosphate, and sulfate, as described in [16]. Samples for Al, Fe and Mn were acidified to pH<2 with HCl, and analyzed at the Brown University Environmental Chemistry Facility with a JY2000 Ultracore ICP Atomic Emission Spectrometer (Horiba, Kyoto, Japan) equipped with a JY AS 421 autosampler and 2400 g mm⁻¹ holographic grating. Details of the MS2 viral transport measurements can be found in Supplemental Methods.

Timeline

The mesocosms received wastewater for 24 months prior to this experiment. The data representing present climate in this study was collected for four months prior to implementation of climate change conditions. Climate change data was collected after the STAs had equilibrated, approximately four months after the change in climate conditions. Estimate of the time required for equilibration of STAs was determined based on the time for recovery of water quality functions following environmental disturbances reported in [20], as well as stabilization of variation in water quality functions following the water table and temperature elevations in our experiment.

Statistics

A non-parametric two-way ANOVA was used to evaluate differences in removal of BOD₅, FCB, total P, and total N as a function of STA type and climate conditions using untransformed data, except for total P, which was transformed using a 1/(n) transform. Means separation was accomplished using the Holm-Sidak method. All statistical tests were performed on averaged replicate data by sampling date collected over four months, and evaluated at $p \leq 0.05$.

RESULTS AND DISCUSSION

Increased moisture and lower O₂ under climate change

Climate change was expected to result in wetter soils with lower O₂ relative to present climate in all three STAs. Water-filled pore space (WFPS) increased under climate change for conventional and shallow narrow STA types at all depths (Fig 3.1). Values of WFPS for P&S ranged from 3%-11% under present climate and increased to

10%-47% under climate change, whereas WFPS increased from 5%-23% to 16%-29% in the shallow narrow STAs under climate change. The concentration of O₂ in soil pores was lower under climate change relative to present climate at all depths (Fig 3.2). Less O₂ and higher WFPS in the STA can have a number consequences for contaminant removal processes, discussed below.

Enhanced BOD₅ removal under climate change may not be beneficial

Climate change resulted in a decrease in the median concentration of BOD₅ in output water from 0.3 to 0.0 mg L⁻¹ for P&S and SND, and remained at 0.0 mg L⁻¹ for GEO (Fig 3.3). The concentration of BOD₅ in output water was significantly different between climate conditions ($p = 0.011$), but not among STA types ($p = 0.699$) (Fig 3.3). Pairwise comparisons of means between STA types were not significant under either climate condition.

Variable BOD₅ removal and perched water table. Variability in the concentration of BOD₅ in output water was higher for GEO under climate change in comparison to present climate, whereas variability in output BOD₅ concentration in SND and P&S was similar between climate conditions. Higher variability of BOD₅ removal in GEO under climate change may be due to an increased probability of developing a perched water table at soil textural discontinuities under climate change [21]. The soil in the shallow narrow mesocosms have a marked textural discontinuity below the infiltrative surface, between the B (silt loam) and C horizons (very gravelly coarse sand). As water moves down the soil profile, capillary suction causes it to collect in the finer textured soil above the discontinuity boundary. This results in a temporary increase in moisture content, limiting O₂ diffusion, and establishing

hypoxic conditions. Once the soil water content is sufficiently high above the textural discontinuity, the force of gravity overcomes capillary suction, and the water drains into the horizon below. The soil textural discontinuity is not a factor in the P&S STAs, where deeper placement of the infiltrative surface was exclusively in the C horizon, below the discontinuity.

Less BOD₅ limits heterotrophic processes. Our results generally support the hypothesis that BOD₅ removal may increase under climate change. Soil microbial communities are carbon limited [22], and BOD₅ is expected to be well removed in the STA. Decomposition of organic carbon in soil is dependent on soil moisture content and temperature [12,23]. Because both soil moisture and temperature increased with climate change, we cannot ascertain the contribution of each variable to increased BOD₅ removal. Nevertheless, greater BOD₅ removal can have important consequences for heterotrophic processes in the STA, such as denitrification, as discussed below.

Release of fecal coliform bacteria increased under climate change

No FCB were detected in output water under present climate (Fig 3.3). In contrast, FCB was detected in output water from all three STA types under climate change, with maximum concentrations of 17, 6, and 20 CFU 100mL⁻¹ for P&S, SND and GEO, respectively. Median output water concentrations were 0.0 CFU 100mL⁻¹ for P&S and GEO, and 0.1 CFU 100mL⁻¹ for SND. Differences were not statistically significant between climate conditions ($p = 0.106$) or among STA types ($p = 0.696$). The presence of FCB in output water was more variable under climate change for all STA types, with greater variability observed in SND and GEO (Fig 3.3).

Wetter soil likely reduced microbial attachment. Unsaturated conditions favor FCB removal in the STA [3,24] by increasing the opportunity for attachment to soil particles. Increased moisture content likely reduced bacterial attachment to soil, resulting in more FCB in output water under climate change. Furthermore, greater bacterial survival has previously been observed in wetter soils [9]. Growth of FCB may also have taken place under climate change, as indicated by higher numbers of FCB in output water from SND and GEO under climate change relative to inputs from SFE on two out of 11 sampling events (data not shown). Others [25,26] have observed the similar survival and propagation of *E. coli* in soil. Generally, bacterial pathogens in soil experience increased mortality with increased temperatures [14,27]. However, our results suggest that the combination of warmer and wetter soils may have enhanced the transport, survival and/or growth of FCB in all STA types.

Temperature likely less important than moisture for FCB removal. A study modeling *E. coli* transport at 20°C and 23°C found greater attenuation at the higher temperature, but predicted lower *E. coli* removal under simulated increases in rainfall leading to wetter soils [27]. Because these two effects were not coupled in the model, and we observed lower FCB attenuation, this would suggest that the degree of soil moisture plays a larger role in bacterial removal than temperature.

Our results suggest the possibility of greater presence of FCB – and thus pathogenic bacteria - in output water, particularly if bacterial growth takes place under climate change. Many pathogenic microorganisms require relatively small doses to cause illness in humans. For example, *E. coli* O157:H7, which produces shiga toxin

and can cause kidney failure and death - requires fewer than 10 cells to cause illness [28], while an infected human will release 10^5 - 10^8 cells in feces [29].

Virus removal unlikely to be impacted by climate change

We determined the effects of climate change on the fate and transport of viruses using MS2 bacteriophage, a surrogate for human viruses [30]. MS2 was not detected in output water from any STA type under present climate or climate change (data not shown). Greater virus transport and survival has been observed in wetter soils [10]; however, virus mortality generally increases with increased temperature [15]. The absence of differences in virus removal between climate conditions and among STA types suggests a common mechanism for viral removal and inactivation under all of these conditions. Viral particles develop a positive charge at pH values below their isoelectric point (pI). The pI of most bacteriophage and animal viruses is < 7.0 [31], and the pH of the soil in the STAs was < 3.5 [16], suggesting that viruses are likely retained on the negatively-charged soil surfaces. This ionic interaction is probably more important than the effects of temperature or soil moisture on the fate and transport of viruses in these STA types.

Acidic soils important for viral removal. The absence of viruses in output water regardless of climate conditions has positive consequences for public health. Enteric viruses can cause illness in humans from ingestion of a single viral particle [32], and the feces of a human infected with rotavirus contains up to 10^7 viral particles [29]. Our results suggest that virus removal in STAs with soil conditions similar to the ones used in our study is unlikely to be affected by climate change.

Effects of climate change on N removal dependent on STA type

The median total N removal was lower under climate change for SND and GEO (Fig 3.4), decreasing from 6% to -11% in SND and from 7% to 3% in GEO, resulting in a net increase in total N concentration in output water relative to present climate conditions. In contrast, the median total N removal for P&S increased, from 14% under present climate to 19%, under climate change (Fig 3.4). The differences in total N removal between climate conditions were not significant ($p = 0.171$), although differences in removal among STA types were ($p = 0.008$). Pairwise comparisons of means between STA types indicated that P&S was significantly different from SND or GEO; however, there was no significant difference in N removal between SND and GEO. Total N removal was more variable under climate change for all STA types, which likely contributed to the absence of a statistically significant effect of climate conditions. There were more events of no net change in N concentration or net increase in total N concentration in output water under climate change (data not shown). Under present climate, 25% (SND) and 100% (GEO and P&S) of the observations reflected net removal of total N. However, 27% (SND) and only 63% (GEO and P&S) of climate change observations resulted in net removal of total N. The frequency of total N leaching in SND was similar between climate conditions, however the concentration of the leaching increased under climate change.

Heterotrophic N removal limited under climate change. Lower availability of organic carbon (Fig 3.3) likely contributed to lower total N removal in SND and GEO under climate change. Heterotrophic denitrification is considered to be the primary mechanism for N removal in the STA [33-35], and requires organic carbon as an

electron donor to produce N_2 and N_2O from nitrate (NO_3^-). The shallow narrow STAs receive SFE, which has a low initial concentration of BOD_5 as a result of passage through an aerobic sand filter (Table 3.1). Greater removal of BOD_5 (Fig 3.3) under climate change in SND and GEO may have limited heterotrophic denitrification in these STAs, particularly if BOD_5 removal takes place closer to the infiltrative surface. In contrast, the P&S drainfield receives STE, which has a higher initial concentration of BOD_5 (Table 3.1), and organic carbon availability may not limit total N removal (Fig 3.4). The leaching of total N in excess of inputs was potentially due to an increase in the frequency of N cycling. In a previous study [21], we presented evidence of internal recycling of N through uptake and re-mineralization of microbial biomass N. Establishment of climate change conditions, and potentially increased incidents of water table perching in SND and GEO, may have caused the rate of internal recycling to increase, increasing the probability of sampling occurring when net leaching of N to output water took place.

Microbial processes other than heterotrophic denitrification can contribute to N removal in the STA, including N_2O production during nitrification [36], N_2 production from anaerobic ammonia oxidation [37], and N_2 production from autotrophic denitrification [38,39]. We have shown evidence for the occurrence of these processes in the STA [21]. Lower available O_2 (Fig 3.2) due to warmer and wetter conditions under climate change would be expected to favor N removal by both autotrophic and heterotrophic processes in all three STAs. However the impact of climate change may have been greater on heterotrophic N removal, resulting from organic carbon limitations in shallow narrow STAs.

Rapid movement of wastewater in STA may limit N removal. We evaluated N removal in the STA using the Damköhler number (D_a) [40], which compares the timescales of transport and reaction rate. Values $D_a < 1$ indicate that elevated rates of transport limit denitrification, and $D_a > 1$ indicate that reactant (e.g. NO_3^-) consumption limits denitrification [41]. This approach has been employed successfully by others to identify the extent to which transport and biochemical reactions control removal of N in groundwater and riparian zones [41,42]. The Damköhler number was calculated using the equation:

$$D_a = kC_0^{n-1}\tau \quad (2)$$

where D_a = Damköhler number (unitless), k = reaction constant, zero-order ($\text{mg L}^{-1} \text{ h}^{-1}$), C_0 = initial concentration of nitrate (mg L^{-1}), n = reaction order (zero order), τ = mean residence time = L/v (h), where L = distance between sample ports (cm) and v = velocity (cm h^{-1}) [40].

Our analysis shows that rapid movement of water through the STA limits denitrification (Fig 3.5), as indicated by values of $D_a < 1$ for all STA types (S3.1 Table). The residence time of the wastewater is 3-4 times greater in P&S than SND and GEO. Furthermore, the value of D_a is lower for SND and GEO, and higher for P&S under climate change relative to present climate, reflecting differences in the reaction rates between the different climate conditions, since the velocity of water remained the same under both climate regimes. Our results suggest that the movement of water through the STA may be too rapid for substantial denitrification to take place, regardless of STA type and climate conditions (S3.1 Table). Improvement of total N

removal may be achieved through slower water movement, which would allow for higher rates of NO_3^- consumption.

Models of N removal need more parameterization. In a model simulation of N removal within our STA mesocosms, Morales et al. [43] predicted increased N removal at 23°C (in comparison to 20°C) under present climate depth to the water table, and higher N removal as the water table was elevated. The poor agreement between the modeled simulation and experimental results suggest that additional factors affected by climate change, such as higher consumption of organic C, need to be incorporated in the model.

Our results suggest that climate change may increase inputs of N to ground water from shallow narrow STAs. Higher inputs of N to groundwater under climate change increases the probability of affecting ecosystem and public health. Eutrophication from excessive inputs of N to saline water bodies may lead to hypoxia and anoxia when microorganisms decompose plant material after death, killing fish and other aerobic organisms. High levels of NO_3^- in ground water may also increase the risk of methemoglobinemia in infants.

Phosphorus removal diminished under climate change

Median total P removal under present climate was close to 100% for SND and GEO, and declined to 71% for SND and 72% for GEO under climate change (Fig 3.4). Median total P removal in P&S also declined from 75% under present climate to 66% under climate change. The differences in total P removal between climate conditions were significant ($p = <0.001$), as well as differences in removal among STA types ($p =$

0.004). Pairwise comparisons of means between STA types indicated that P&S was significantly different from SND or GEO; however, SND and GEO did not have different removal. As was the case for removal of other wastewater constituents, we observed higher variability in total P removal under climate change than under present climate in all STA types (Fig 3.4).

Reduction of metal-P complexes mobilize P. Our results support the hypothesis that total P removal may diminish under climate change for all STA types. The mechanism for this effect may involve lower availability of O₂ in the STA under climate change. Limited O₂ availability likely lead to reduction and increased solubility of redox-active metals (Fe and Mn) in soil involved in forming insoluble precipitates with phosphate. Along with formation of precipitates with Al oxides, this is thought to be the primary mechanism for total P removal in the STA [11,16]. Reduction of Fe and Mn increases their solubility, which not only releases phosphate bound to Fe and Mn oxides into the dissolved phase, but also results in a decrease in the number of metal oxide sites available for reaction with – and retention – of phosphorus. Dissolution of Al at the acidic pH found in the STA (< 3.5) may have also contributed to P leaching [16], however, the pH of the output water, and likely STA soil, was similar under both climate conditions.

Abiotic mechanisms appear more important for P retention. To differentiate between potential abiotic mechanisms affecting P removal, we plotted the concentration of dissolved Fe, Mn and Al in output water versus the concentration of total P for all three STA types under present climate and climate change (Fig 3.6). The closer the slope of the line of metal concentration vs. P in solution is to 1 (indicating

stronger coupling) – which describes the stoichiometry of the metal-P complexes – the more likely it is that P was released from complexes formed with that metal. Under present climate, the slope of the line for all three metals is considerably less than 1, indicating that dissolution of metal-P complexes was not responsible for release of total P to output water (Fig 3.6). In contrast, under climate change the slope of the line for Fe and Mn is much closer to 1, suggesting that reduction of these metals became more important for total P release under climate change. Because Al and total P in output water were not strongly coupled (Fig. 3.6), this suggests that climate change did not strongly influence this mechanism.

We note that the concentration of these metals in output water did not increase substantially under climate change. It appears that, rather than a larger amount of metal becoming soluble under climate change, a larger fraction of the Fe and Mn minerals involved in complexing P were reduced and rendered soluble.

Our results suggest that the concentration of total P in output water from the STA may increase under climate change. The resulting higher levels of total P in groundwater can eventually lead to increased eutrophication of fresh water bodies. In addition to the detrimental effects of eutrophication on aquatic organisms, it may also lead to public health concerns related to production of trihalomethanes (THM), carcinogenic compounds produced from chlorination of drinking water [44]. Algal blooms can also result in the production of human toxins that prevent use of surface water for drinking [45,46].

Whole system evaluation

To compare the performance of the treatment trains that include conventional and shallow narrow STAs under present climate and climate change, we estimated contaminant removal over the course of a year (S3.2 Fig and Fig 3.7). We used mean values for contaminant concentrations in input water (Table 3.1) and, for treatment trains including SND and GEO, assumed that contaminant removal rates in the sand filter did not change with climate change. At the system scale, more BOD₅ was released from a treatment train with a conventional STA than from treatment trains with shallow narrow STAs under both climate conditions likely due to sand filter pre-treatment. A greater number of FCB were present in output water from systems with a shallow narrow STAs than a system with a conventional STA under climate change conditions, whereas complete FCB removal was observed under present climate in all three systems. A higher mass of total P was released from treatment trains with a conventional STA than from treatment trains with shallow narrow STAs under both climate conditions. A greater mass of total N was present in output water from systems with a conventional STA under present climate, however, more total N was present in output water from systems with shallow narrow STAs under climate change (Fig 3.7). Because the shallow narrow systems are not designed to enhance N removal, use of alternative OWTS with advanced N removal components should improve N removal rates under any climate conditions.

CONCLUSIONS

Our results indicate that climate change can affect contaminant removal, with effects dependent on the contaminant and STA type. Removal of FCB, total P and

total N in shallow narrow STAs diminished under climate change conditions. In contrast, total N removal in conventional STAs improved. Viral pathogens and BOD₅ were well removed under climate change, suggesting that OWTS were more resilient with respect to these contaminants.

Although conditions in the field may diverge from those in the laboratory, our experiment allowed us to make direct comparisons between present climate and climate change among different STA types. We recognize that systems installed under field conditions have more performance variability than systems evaluated under laboratory conditions [47]. Despite the rapid transformation to climate change conditions in our study, our results provide potential long-term consequences of climate change. Warming the entire STA, as opposed to only the near surface under field conditions, enabled us to make direct observations between the two temperature conditions at all depths in the soil profile. While the length of our study was relatively short, the limited duration prevented extreme temporal variation in the STA microbial communities between climate conditions.

Although the response of abiotic and biotic components in OWTS to differing temperature and moisture conditions may not be linear, our results demonstrated the potential effects of climate change on different types of OWTS. This study provides regulators with a starting point for future planning as well as providing an impetus for designing improvements for OWTS technologies. Addition of carbon amendments and more effective pretreatment components to OWTS treatment trains will likely make them more robust to both short and long term changes in climate.

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TABLES

Table 3.1 Characteristics of septic tank effluent (STE) and sand filter effluent (SFE) used in our study under present climate (n=8 samples) and climate change (n=11 samples). Values are means \pm standard deviation. All units are mg L⁻¹ except for pH, electrical conductivity (μ S), fecal coliform bacteria (CFU 100 mL⁻¹), and collection temperature (°C).

Analyte	STE		SFE	
	Present climate	Climate change	Present climate	Climate change
ph	6.3 \pm 0.2	6.5 \pm 0.2	3.9 \pm 0.9	4.6 \pm 0.5
Dissolved O ₂	0.0 \pm 0.0	0.0 \pm 0.0	2.4 \pm 0.6	3.2 \pm 1.3
BOD ₅	219 \pm 61	140 \pm 79	11 \pm 7.3	6.1 \pm 8.6
Electrical conductivity	786 \pm 47	620 \pm 146	615 \pm 85	422 \pm 120
Fecal coliform bacteria	1.4 $\times 10^6$ $\pm 1.8 \times 10^6$	1.1 $\times 10^5$ $\pm 1.2 \times 10^5$	4.2 $\times 10^3$ $\pm 6.7 \times 10^3$	1.6 $\times 10^1$ $\pm 2.7 \times 10^1$
Total N	67 \pm 8.0	52 \pm 15	58 \pm 8.0	44 \pm 11
NH ₄ -N	50 \pm 7.0	36 \pm 15	10 \pm 4.8	5.7 \pm 2.9
NO ₃ -N	0.02 \pm 0.04	0.02 \pm 0.02	40 \pm 8.0	24 \pm 8.7
Total P	9.1 \pm 0.6	7.4 \pm 2.1	7.8 \pm 1.2	6.3 \pm 1.6
PO ₄ -P	6.9 \pm 0.4	5.70 \pm 1.7	50 \pm 0.4	4.7 \pm 1.2
SO ₄ -S	9.3 \pm 1.3	8.5 \pm 2.4	15 \pm 2.8	13 \pm 3.9
Collection temperature	20 \pm 2.0	12 \pm 6.1	20 \pm 2.2	11 \pm 6.8

FIGURES

Fig 3.1 Water-filled pore space (WFPS) in conventional (P&S) and shallow narrow (SND and GEO) soil treatment areas under present climate and climate change. Values represent the average WFPS over 24 h at each depth.

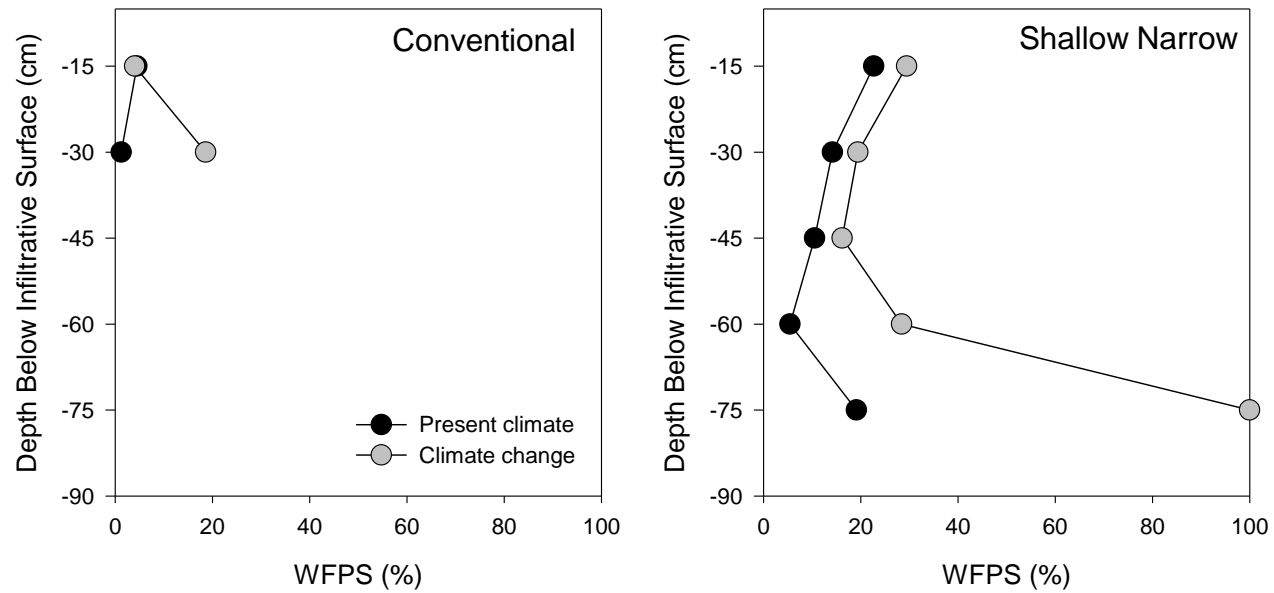


Fig 2. Soil pore O_2 concentration under present climate and climate change for shallow narrow (SND and GEO) and conventional (P&S) soil treatment areas. Values are means ($n = 8-11$ samples); error bars represent one standard deviation for a single mesocosm over four months.

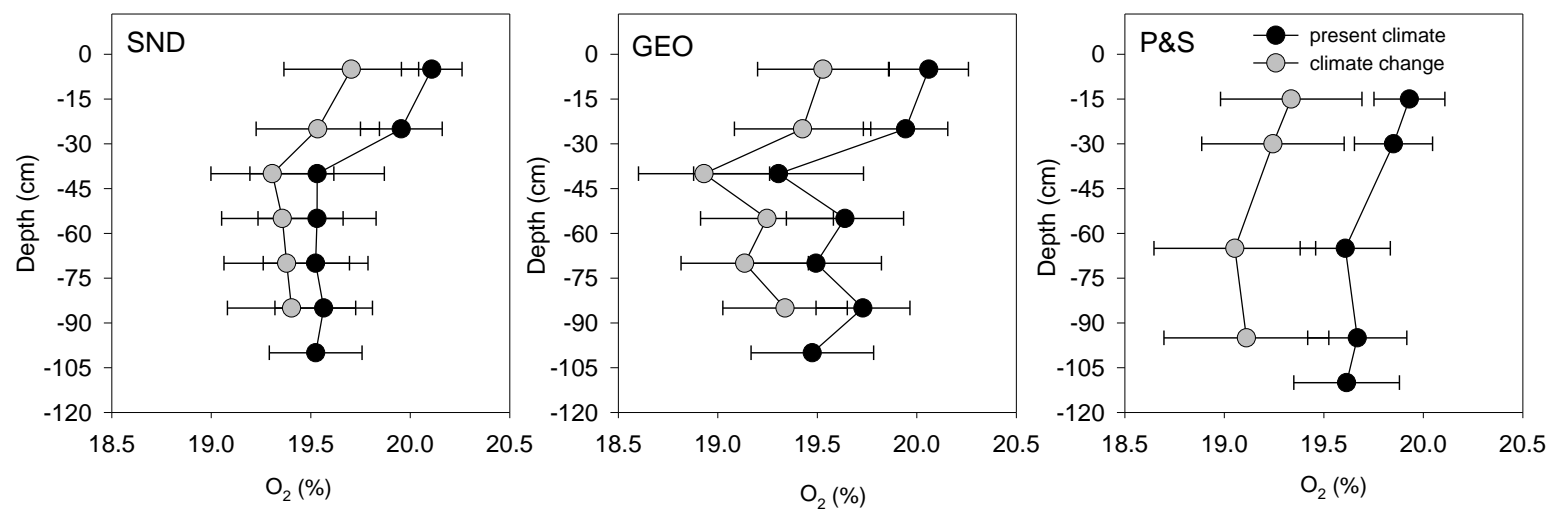


Fig 3.3 Concentration of biochemical oxygen demand (BOD₅) (top) and fecal coliform bacteria (FCB) (bottom) in output water under present climate and climate change for shallow narrow (SND and GEO) and conventional (P&S) soil treatment areas. Values are averages of three replicates by sampling date. Boxes represent the median and interquartile range, whiskers represent the 10th and 90th percentiles, and dots represent values outside the 10th and 90th percentiles of the sample dates.

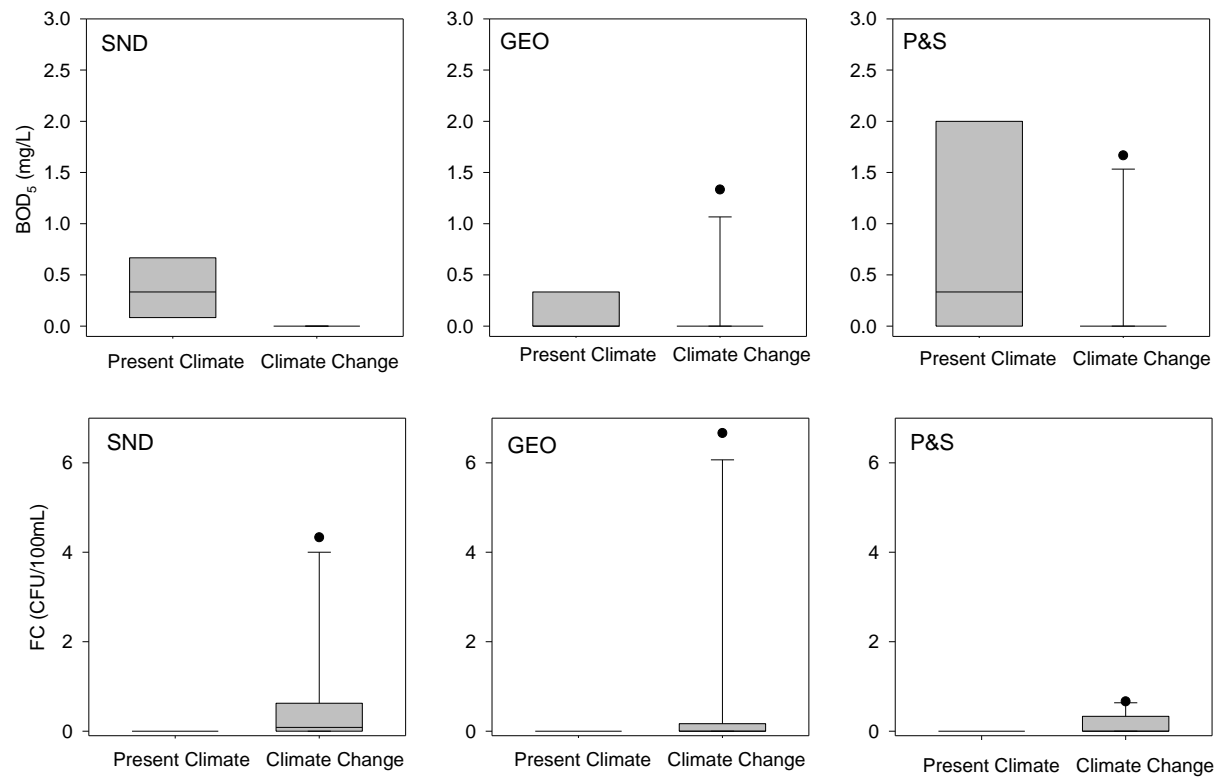


Fig 3.4 Total nitrogen (top) and total phosphorus (bottom) removal under present climate and climate change for shallow narrow (SND and GEO) and conventional (P&S) soil treatment areas. Values are averages of three replicates by sampling date. Boxes represent the median and interquartile range, whiskers represent the 10th and 90th percentiles, and dots represent values outside the 10th and 90th percentiles.

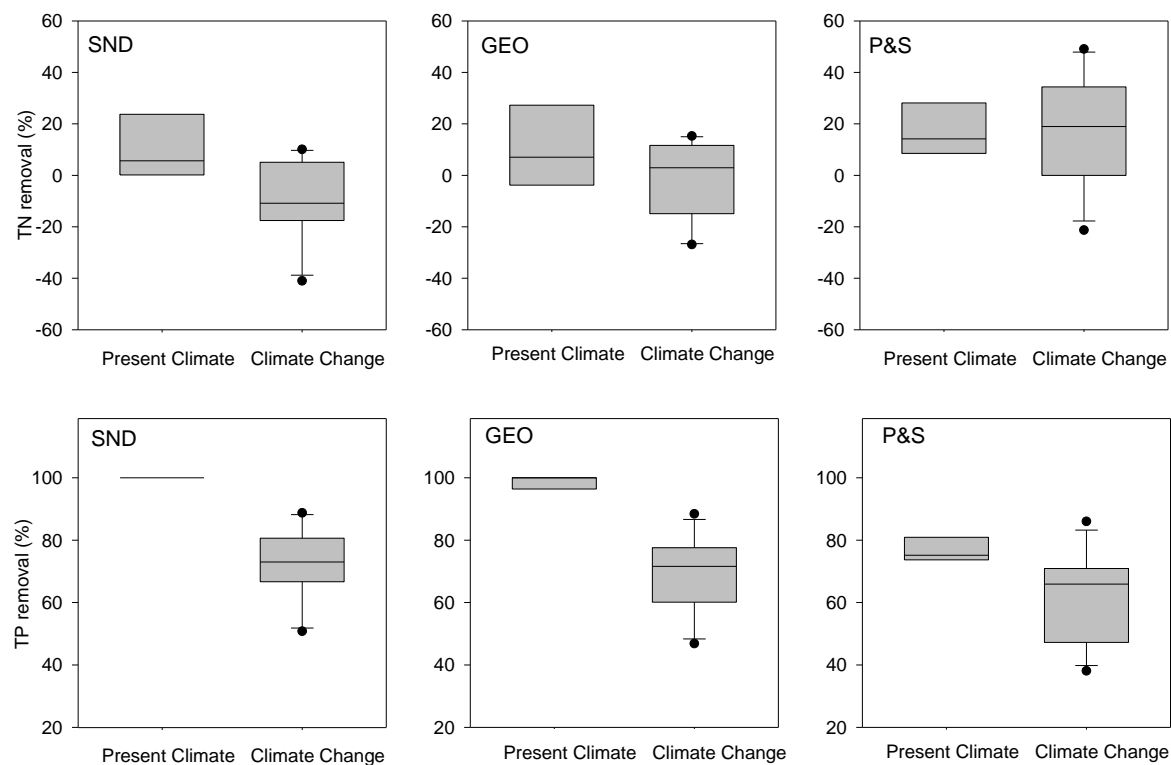


Fig 5. Damköhler number (D_a) values under present climate (PC) and climate change (CC) for N removal in shallow narrow (SND and GEO) and conventional (P&S) soil treatment areas.

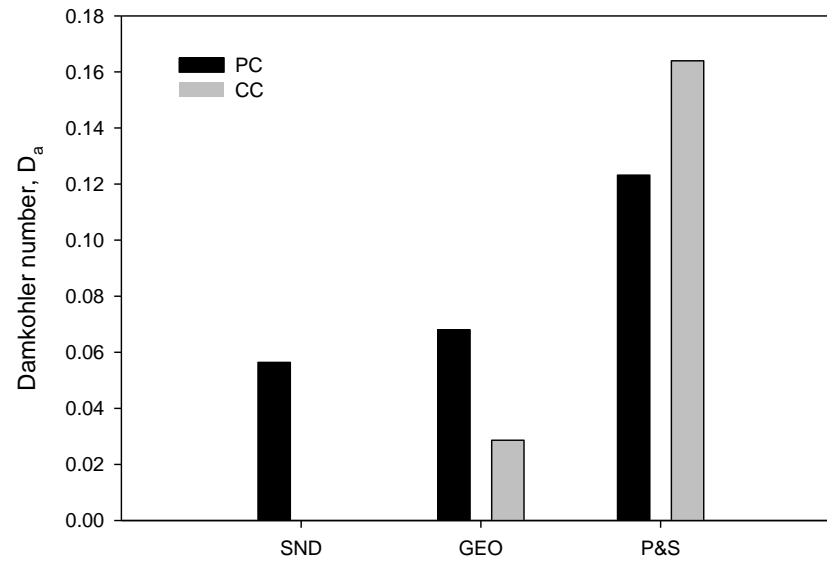


Fig 3.6 Concentrations of Fe, Al and Mn vs. total P (TP) in output water from all three STA types (n=8). Dashed line describes the stoichiometry of metal-P complexes.

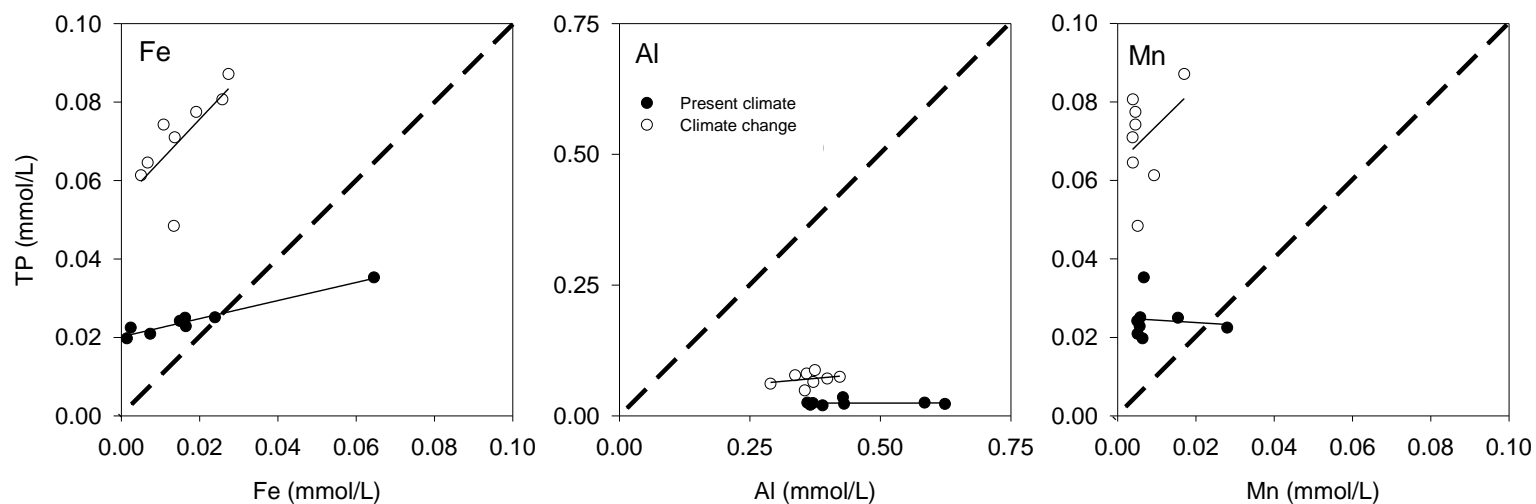
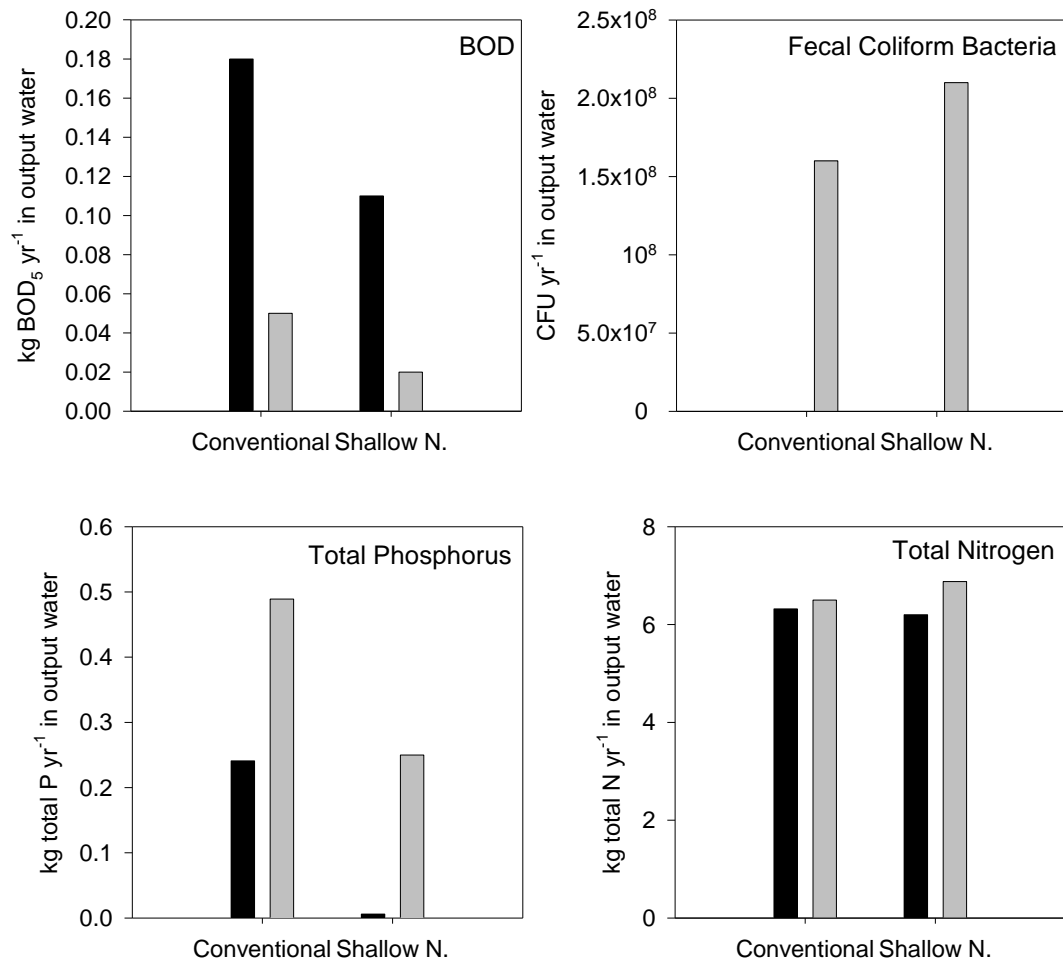


Fig 3.7 Yearly estimates of mass of BOD₅, total P and total N, and number of fecal coliform bacteria released to groundwater from treatment trains that include P&S and shallow narrow (SND & GEO) STAs under present climate (dark bars) and climate change (light bars).

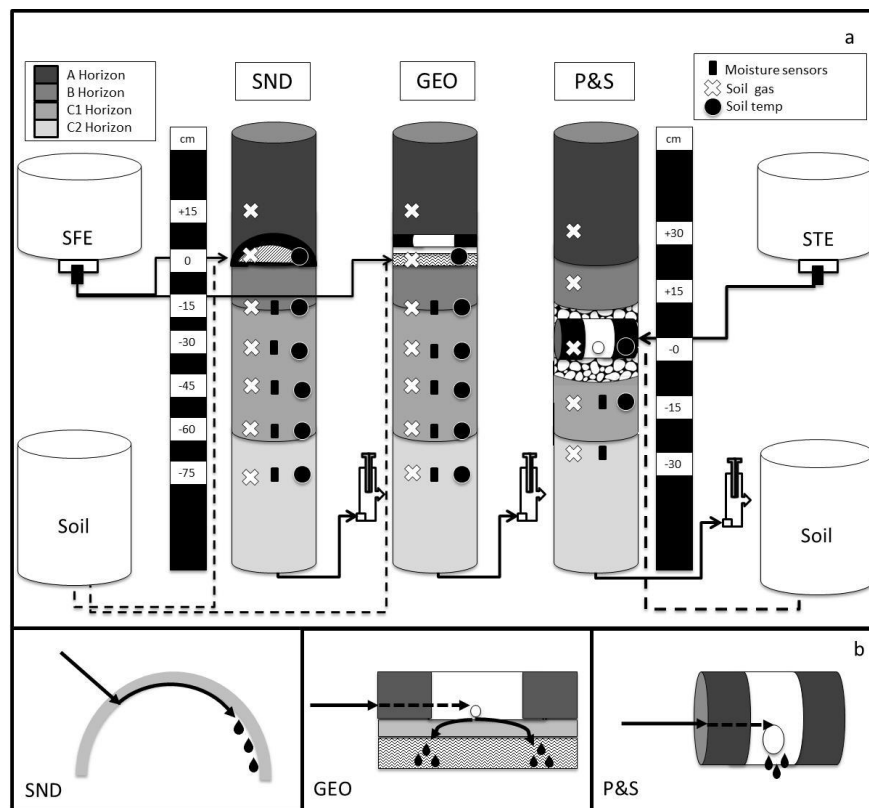


SUPPORTING INFORMATION

Methods for MS2 bacteriophage removal experiment. ATCC® 15597-B1™ MS2 bacteriophage was propagated by addition of 0.5 mL seed to a 6 h culture of ATCC® 15597™ *Escherichia coli* strain C3000 (*E. coli*) grown in ATCC® Medium 271 at 37°C. Following cell lysis, MS2 was enumerated using the soft agar overlay method (Adams, 1950*). MS2 was added to an aliquot of wastewater, bringing the final concentration to 8×10^7 pfu mL⁻¹. Sodium chloride was added to the virus wastewater mixtures as a conservative tracer to a final concentration of 5000 mg L⁻¹ NaCl. The virus and wastewater mixtures were added to the STA in a 200 mL-dose to P&S over 1.5 h, and in a 200 mL-dose to SND and GEO in ~4.5 doses of 42 mL over 2.25 h. Output water was analyzed daily for MS2 bacteriophage plaques using the soft agar overlay method for 10 days.

* Adams M H. Bacteriophages. Interscience, New York. 1950.

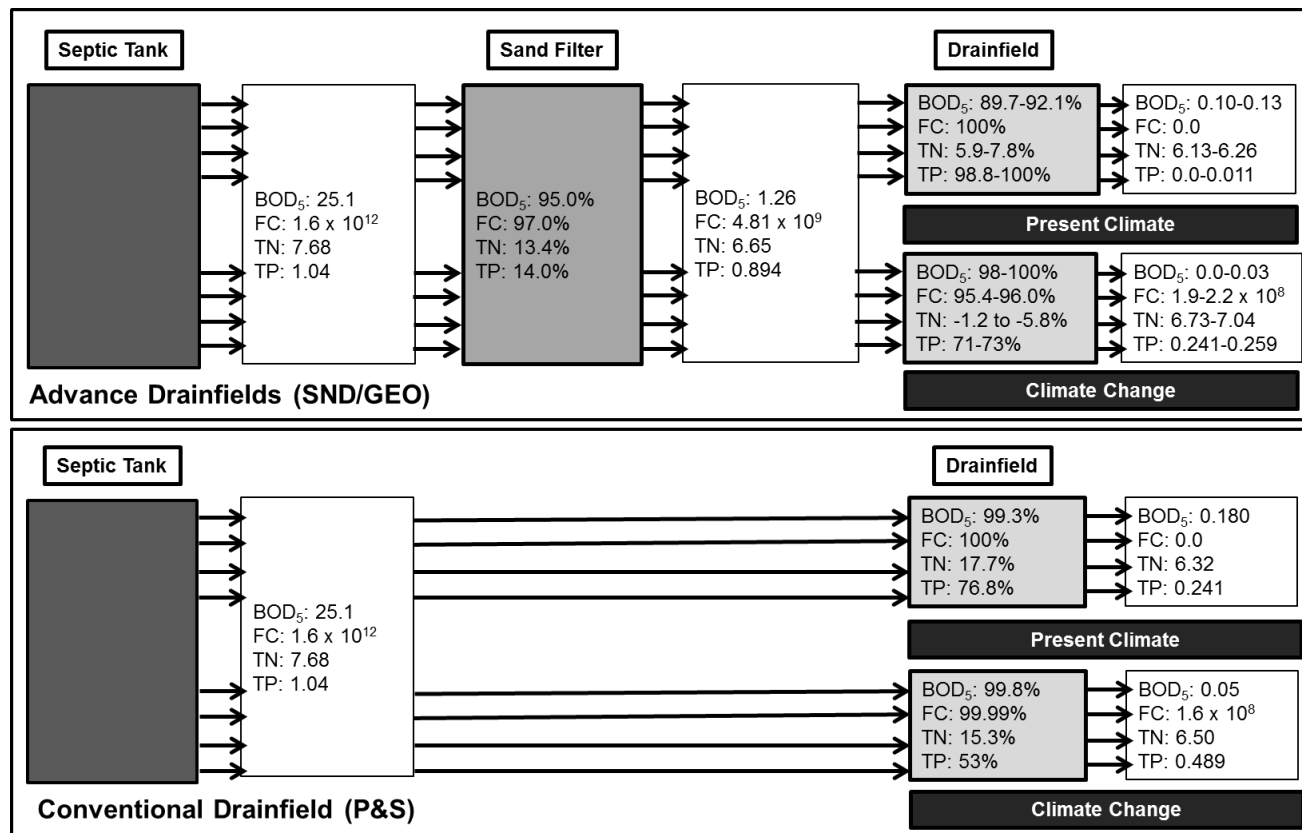
S3.1 Fig. (A) Schematic diagram of soil mesocosms representing a shallow narrow drainfield (SND), GeoMat® (GEO), and pipe and stone (P&S) soil treatment areas (STAs). The wastewater input to SND and GEO was sand filter effluent (SFE), whereas the P&S received septic tank effluent (STE). The approximate location of soil horizons, ports for gas sampling, and moisture and temperature probes are indicated. Water exits the mesocosms through a hanging water column device used to adjust the height of the water table. The atmosphere in the infiltrative area is connected to a 30-cm soil column. (B) Detailed schematic diagram of the SND, GEO and P&S delivery devices. Diagrams are not to scale. Heating cables were wrapped around mesocosms, covered with insulation, and connected to a digital thermostat to control soil temperature.



S3.1 Table. Measured parameters used to calculate the Damköhler Number (D_a) under present climate (PC) and climate change (CC) conditions for nitrate removal within shallow narrow (SND/GEO) and conventional (P&S) soil treatment areas

Parameter	SND		GEO		P&S	
	PC	CC	PC	CC	PC	CC
Length, L (cm)	102	102	102	102	56	56
Velocity, v (cm h ⁻¹)	1.7	1.7	1.5	1.5	0.3	0.3
Rate constant, zero order, k_0 (mg L ⁻¹ h ⁻¹)	0.047	0.0	0.05	0.021	0.033	0.044
Initial nitrate concentration, C_0 (mg L ⁻¹)	50	50	50	50	50	50
Damköhler number, D_a	0.056	0.0	0.068	0.029	0.12	0.16

S3.2 Fig. Estimates of field-scale mass loading from septic tank, sand filter and soil-based treatment for an advanced system with a shallow narrow (SND) or GeoMat® (GEO) soil treatment area, and for a conventional system with a pipe and stone (P&S) soil treatment area. Removal values (%) are for the previous step in the treatment train. Units are kg yr⁻¹ except for fecal coliform bacteria (FCB), which are CFU yr⁻¹.



CONCLUSIONS

My results show that the P&S, SND and GEO drainfields are equally effective for removal of BOD₅, FCB, and total P under present climate conditions. Furthermore, similar mechanisms (e.g. microbial decomposition, filtration, absorption, precipitation) for water quality renovation appear to be at work in conventional and shallow narrow STAs, despite differences in placement in the soil profile, physical and chemical properties of the receiving soil, and separation distance from the water table. In contrast, N removal differed among the STA types, with 12.0% for P&S, 4.8% for SND and 5.4% for GEO. When the whole treatment train (except the septic tank) is considered, advanced treatment technologies incorporating a shallow narrow STA exceeded the N removal capabilities of a conventional STA alone.

With respect to N removal, we found both conventional and shallow narrow STAs produced $100 - 1000 \times$ more N₂ than N₂O, despite the differences in composition of wastewater inputs, placement of the STA within the soil profile, and soil physicochemical conditions. This suggests that both autotrophic and heterotrophic processes contribute to N losses in all STA types, with wastewater composition, and availability of electron donors and organic carbon controlling N removal in the STA.

The results of this study demonstrated that climate change did affect the capacity of OWTS to treat contaminants, with the extent of removal dependent on the type of contaminant and the type of STA. Climate change conditions resulted in diminished removal of FCB and total P for all STA types. The extent of total N removal was improved in conventional STAs from 14% under present climate to 19% under climate change, while total N removal decreased in shallow narrow STAs from 6-7% under present climate to less than 3.0%. However, BOD₅ and viral pathogens were well removed under climate change, indicating that

given similar soil conditions, removal of these contaminant are not likely to be effected by climate fluctuations.

Although conditions in the field may diverge from those in the laboratory, this experiment allowed direct comparisons among drainfield types. While this study was limited in its ability to completely replicate the technology dosing configurations, using intact soil mesocosms allowed us to control for variables that may preclude direct comparisons of results, such as differences in temperature, soil properties, exogenous inputs of nutrients, pathogens and water, and wastewater composition that would have occurred under field conditions. I chose not to grow vegetation on the STAs as this would have interfered with gas fluxes, however, at most this could account for an additional ~2% N-removal in the shallow narrow STAs (Holden et al., 2004a). The poorly buffered acidic soils found in Rhode Island differ will differ from soils in other parts of the world, however, as many soils have lower pH, my result should be relevant to many regions.

My results suggest that we need to be proactive with respect to technological improvements and policy changes that will make OWTS more resilient to climate change. Incorporate risk-based best management practices into siting and design policy to make OWTS more climate change resilient. This may include mapping and establishing priorities for at-risk areas, increasing vertical and horizontal setbacks and buffers, and requiring soil moisture management through use of timed-dosing and flow equalization. The addition of organic carbon within the treatment train to enhance heterotrophic processes such as denitrification may be an effective climate change adaptation strategy. Greater unsaturated separation distances in the STA will likely aid in removal of total P and pathogens. Given the variable treatment observed within the STA, and its sensitivity to environmental perturbations, enhanced removal of contaminants (through the use of bioreactors, advanced treatment technologies, etc.) before the STA would likely be beneficial. This study also noted poorer

removal of contaminants from more rapid movement of wastewater through the STA. While pretreatment has been utilized as a method to allow a greater volume of wastewater to infiltrate over a shorter time interval, thus allowing a reduction in the STA footprint, this may not be the most effective treatment pathway under climate change conditions. Furthermore, I suggest that regulators implement a monitoring program for OWTS, especially for technologies claiming enhanced contaminant removal, to directly test if greater removal is occurring, and how to optimize treatment. Industry is encouraged to adapt and improve existing advanced treatment technologies and develop new one that are resilient to climate variability.

While my study provides a starting point for evaluating climate change effects on OWTS, field studies will be needed, including those that measure removal in the soil. Direct field comparisons of differing contaminant removal methodologies (e.g. pretreatment technologies vs. passive soil amendments) is the best procedure for optimization of technology design. Additionally, microbial community analyses, and a better understanding of gene expression and functional protein production will provide more insights into mechanistic drainfield processes.

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